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(54) Title: USE OF HEPARANASE TO IDENTIFY AND ISOLATE ANTI-HEPARANASE COMPOUND			
(57) Abstract Purified heparanase having activity of greater than 20 units/ μ g protein, preferably greater than 50 units heparanase activity per μ g protein, is described. The use of heparanase for screening for anti-heparanase compounds is also described. In addition, the use of the high potency heparanase to accelerate wound healing or its use as an immobilized heparanase filter connected to extracorporeal devices to degrade heparin and neutralize its anticoagulant properties during surgery is disclosed.			

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USE OF HEPARANASE TO IDENTIFY AND ISOLATE ANTI-HEPARANASE
COMPOUND

FIELD OF INVENTION

The present invention discloses the use of mammalian heparanase, preferably
5 recombinant heparanase, for screening for anti-heparanase compounds. More particularly, the
present invention provides a method of selecting IHA (Inhibitors of Heparanase Activity). In
addition, the present invention provides a purified heparanase, particularly suitable for use to
identify and isolate anti-heparanase compounds as well as for other known uses of heparanases,
such as its use to accelerate wound healing or its use as an immobilized heparanase filter
10 connected to extracorporeal devices to degrade heparin and neutralize its anticoagulant properties
during surgery.

BACKGROUND OF THE INVENTION

Elevated heparanase activity has been documented in mobile, invasive cells. Examples
include; invasive melanoma, lymphoma, mastocytoma, mammary adenocarcinoma, leukemia,
15 and rheumatoid fibroblasts. This activity has also been documented in non-pathologic
situations involving the migration of lymphocytes, neutrophils, macrophages, eosinophils and
platelets. An inhibitor of heparanase would therefore broadly influence the invasive potential of
these diverse cells.

Inhibition of heparan sulfate degradation would also inhibit the release of bound growth
20 factors and other biologic response modifiers that would, if released, fuel the growth of adjacent
tissues and provide a supportive environment for cell growth (Rapraeger, et al., *Science* 252:
1705-1708, 1991). Inhibitors of heparanase activity would be of value in the treatment of
arthritis, vascular restenosis, tumor growth and progression, and fibro-proliferative disorders.

Until now, the obstacles to designing a screening assay to find inhibitors of mammalian
25 heparanase have been the unavailability of a mammalian heparanase that is purified to apparent
homogeneity and the lack of information about the amino acid sequence or the 3-dimensional
structure of the enzyme. Without the amino acid sequence, it has not been possible to produce
recombinant mammalian heparanase to be used in large volume screening efforts. Knowledge
of the tertiary and quaternary structures would facilitate rational design of IHA. This report
30 overcomes obstacles relating to the sequence of the heparanase, and also provides a model for
higher-order structure.

Heparanase refers to a mammalian enzyme which can degrade heparin proteoglycans
(HPG) and/or heparan sulfate proteoglycans (HSPG).

Heparanase activity in mammalian cells is well known. It is found in various melanoma
35 cells (Nakajima, et al., *Cancer Letters* 31: 277-283, 1986), mammary adenocarcinoma cells
(Parish, et al., *Int. J. Cancer*, 40: 511-518, 1987), leukemic cells (Yahalom, et al., *Leukemia*

Research 12: 711-717, 1988), mast cells (Ogren and Lindahl, *J. Biol. Chem.* 250: 2690-2697, 1975), macrophages (Savion, *et al.*, *J. Cell. Physiol.*, 130: 85-92, 1987), mononuclear cells (Sewell, *et al.*, *Biochem. J.* 264: 777-783, 1989), neutrophils (Matzner, *et al.*, *J. Leukocyte Biology* 51: 519-524, 1992), T-cells (Vettel, *et al.*, *Eur. J. Immunol.* 21: 2247-2251, 1991),
5 platelets (Haimovitz-Friedman, *et al.*, *Blood* 78: 789-796, 1991), endothelial cells (Godder, *et al.*, *J. Cell Physiol.* 148: 274-280, 1991), and placenta (Klein and von Figura, *BBRC* 73: 569, 1976).

WO 91/02977, incorporated herein by reference, describes a substantially, but partially, purified heparanase produced by cation exchange resin chromatography and the affinity
10 absorbent purification of heparanase-containing cell extract. WO 91/02977 also describes a method promoting wound healing utilizing compositions comprising a "purified" form of heparanase.

Others have proposed the use of purified bacterial heparanase, immobilized onto filters and connected to extracorporeal devices, to degrade heparin and neutralize its anticoagulant
15 properties post surgery (Freed, *et al.*, *Ann. Biomed. Eng.* 21: 67-76, 1993).

U.S. Patent 4,882,318 describes heparanase-inhibiting compositions for preventing tumor metastasis.

Haimovitz-Friedman *et al.* (*Blood* 78: 789-796, 1991) describe an assay for heparanase activity that involves the culturing of endothelial cells in radiolabeled $^{35}\text{SO}_4$ to produce
20 radiolabeled heparan sulfate proteoglycans, the removal of the cells which leaves the deposited extracellular matrix that contains the ^{35}S -HSPG, the addition of potential sources of heparanase activity, and the detection of possible activity by passing the supernatant from the radiolabeled extracellular matrix over a gel filtration column and monitoring for changes of the size of the radiolabeled material that would indicate that HSPG degradation had taken place. This assay
25 does not have the capability for large-scale screening of inhibitors.

Nakajima *et al.* (*Anal. Biochem.* 196: 162-171, 1986) describe a solid-phase substrate for the assay of melanoma heparanase activity. Heparan sulfate from bovine lung is chemically radiolabeled by reacting it with [^{14}C]-acetic anhydride. Free amino groups of the [^{14}C]-heparan sulfate were acetylated and the reducing termini were aminated. The [^{14}C]-heparan sulfate was
30 chemically coupled to an agarose support via the introduced amine groups on the reducing termini. This substrate is limited in that it is an extensively chemically modified form of naturally occurring heparan sulfate.

Khan and Newman (*Anal. Biochem.* 196: 373-376, 1991) describe an indirect assay for heparanase activity. In this assay, heparin is quantitated by its ability to interfere with the color
35 development between a protein and the dye Coomassie brilliant blue. Heparanase activity is

detected by the loss of this interference. This assay is limited in use for screening because it is so indirect that other non-heparin compounds could also interfere with the protein-dye reaction.

The CXC chemokine family (also called the intercrine α family) is one branch of the supergene "intercrine" cytokine family (Oppenheim, *Ann. Rev. Biochem.* 9: 617-648, 1991).

- 5 It's members include platelet factor 4, platelet basic protein and derivatives, γ IP-10, gro(α, β, γ), NAP-1/interleukin-8, mig, and ENA-78 (for review, see Miller and Krangel, *Critical Reviews in Immunology* 12: 17-46, 1992). The other branch, the CC chemokines or intercrine- β family, includes MIP1 α , MIP1 β , JE/MCP-1, RANTES, and MCAF. All members of both branches of this chemokine family characteristically are basic heparin-binding polypeptides, display
- 10 molecular weights between 8 and 11 kD, share 20 - 50% homology, and function broadly in pathologic situations characterized by inflammation and tissue remodeling.

- The proteolytically processed forms of platelet basic protein include CTAP-III, β -thromboglobulin, and NAP-2. β -thromboglobulin (Moore, *et al.*, *Biochim. Biophys. Acta.* 379: 360-369, 1975) and CTAP-III (Castor, *et al.*, *Arthritis Rheum.* 20: 859-868, 1977), were
- 15 originally isolated from activated supernatants or lysates from outdated platelets. Using the techniques of subcellular fractionation and radioimmunoassay, β -thromboglobulin was identified as an α -granule protein that could be released upon activation (Kaplan, *et al.*, *Blood* 53: 604-618, 1979). Platelet basic protein itself was later isolated from fresh platelets, megakaryocytes, and HEL cells, an immortal human erythroleukemia cell line (Holt, *et al.*, *Biochemistry* 25:
- 20 1988-1996, 1986; Holt, *et al.*, *Exp. Hematol.* 16: 302-306, 1988). Walz and Baggiolini isolated the processed form of NAP-2 from platelet-containing cultures of stimulated mononuclear cells (Walz, *et al.*, *J. Exp. Med.* 170: 1745-1750, 1989).

- Material labeled as β -thromboglobulin is commercially available from Calbiochem, San Diego, CA (Cat. # 605165), Celsus Laboratories, Cincinnati, OH (Cat. # 41705), and
- 25 Haematologic Technologies, Essex Jct., VT (Cat. # HBTG-02100). The inventors have determined, by using the "Purification Assay," that the commercial preparation have heparanase activity at a level of 0.075 units/ μ g. This activity is below the level of 1 unit/ μ g needed for the screening of anti-heparanase compounds in accordance with the assay of the subject invention.

- U.S. Patent 4,897,348 describes recombinant materials and methods for producing
- 30 human connective tissue-activating peptide-III (CTAP-III) and analogs thereof.

- Transglutaminases catalyze the posttranslational modification of proteins by transamidation of available glutamine residues. This action results primarily in the formation of epsilon-(gamma-glutamyl)lysine cross-links (Greenberg, *et al.*, *FASEB J.* 5: 3071-3076, 1991). This posttranslational modification has been reported to dramatically alter the action of some
- 35 small proteins. For example, a transglutaminase produces a glutamine-lysine cross-link in the 13

kD phospholipase A₂ and increases its specific enzymatic activity (Cordella-Miele, *et al.*, *J. Biol. Chem.* 265: 17180-17188, 1990). A transglutaminase cross-links another small molecule, interleukin-2, and converts its activity to one that is cytotoxic to mature oligodendrocytes (Eitan and Schwartz, *Science* 261: 106-108, 1993). The glutamine-lysine cross-link in a protein would result in the loss of overall positive charge for that protein. The transglutaminases are optimally active and generally used under reducing conditions such as dithiothreitol. The concept that glutamine-lysine cross-linking alters the activity of these small proteins may be applicable to other small molecules as well.

SUMMARY OF THE INVENTION

The present invention discloses a method of screening for compounds having anti-heparanase activity (AHA compounds), i.e. inhibitors of heparanase activity (IHA), comprising the steps of: contacting a potential AHA compound with radiolabeled heparin/heparan sulfate and heparanase for a time and under such conditions sufficient to allow for inhibition of heparanase activity; detecting inhibition of heparanase activity; and selecting compounds that inhibit heparanase activity. The present invention also discloses the amino acid sequence identity of the heparanase that has been purified to homogeneity by chromatography under reducing conditions. Identification of the amino acid sequence of the protein which contains heparanase activity is crucial for the production of recombinant mammalian heparanase.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a purified heparanase, and a method for producing it. The heparanase so produced has an activity of greater than 20 units/ µg protein, preferably greater than 50 units heparanase activity per µg protein (1 unit = 1% cpm < 30 K/hr using the "Purification Assay" (Example 2, Part D).

In addition, the present invention provides recombinant heparanase and a means for producing it. The term "purified heparanase" as used in the specification and claims includes the recombinant heparanase as described in the subject application. The recombinant heparanase of the subject invention can be used for the same purposes and in the same manner as the purified heparanase.

The purified heparanase of the present invention has an isoelectric point of less than 5.5 (preferably about 4.8 - 5.1) and preferably is activated by treatment with transglutaminase using reducing conditions.

The recombinant heparanase of the present invention has an isoelectric point of less than 5.5 (preferably about 4.8 - 5.1), and is isolated under reducing conditions and is activated by treatment with transglutaminase.

Suitable transglutaminases that may be used for this purpose include Activated Factor XIIIa, guinea pig liver transglutaminase, epidermal transglutaminase, keratinocyte

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transglutaminase, and tissue transglutaminase.

The heparanase of the present invention has the amino acid sequence (SEQ. ID. NO: 1) of:

```

5  Asn Leu Ala Lys Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala
    5              10              15

    Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys
        20              25              30

10  Asn Ile Gln Ser Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn Gln
    35              40              45

    Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp
        50              55              60

15  Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly
    65              70              75              80

    Asp Glu Ser Ala Asp
        85
  
```

encoded by the cDNA sequence (SEQ ID NO: 2) of:

```

1  AACTTGGCGA AAGGCAAAGA GGAAAGTCTA GACAGTGACT TGTATGCTGA
51 ACTCCGCTGC ATGTGTATAA AGACAACCTC TGGGAATTCAT CCCAAAAACA
101 TCCAAAGTTT GGAAGTGATC GGGAAAGGAA CCCATTGCAA CCAAGTCGAA
25 151 GTGATAGCCA CACTGAAGGA TGGGAGGAAA ATCTGCCTGG ACCCAGATGC
201 TCCCAGAATC AAGAAAATTG TACAGAAAAA ATTGGCAGGT GATGAATCTG
251 CTGAT
  
```

which corresponds to the cDNA sequence and derived amino acid sequence of CTAP-III. See Wenger et al., *Blood*, 73: 1498-1503, 1989.

30 In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 3) of:

```

    Ser Ser Thr Lys Gly Gln Thr Lys Arg Asn Leu Ala Lys Gly Lys Glu
        5              10              15

35  Glu Ser Leu Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile
    20              25              30

    Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser Leu Glu Val
        35              40              45

40  Ile Gly Lys Gly Thr His Cys Asn Gln Val Glu Val Ile Ala Thr Leu
    50              55              60

    Lys Asp Gly Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys
45  65              70              75              80
  
```

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Lys Ile Val Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala Asp
85 90

encoded by the cDNA sequence (SEQ ID NO: 4) of:

1 TCCTCCACCA AAGGACAAAC TAAGAGAAAC TTGGCGAAAG GCAAAGAGGA
5 51 AAGTCTAGAC AGTGACTTGT ATGCTGAACT CCGCTGCATG TGTATAAAGA
101 CAACCTCTGG AATTCATCCC AAAAACATCC AAAGTTTGGA AGTGATCGGG
151 AAAGGAACCC ATTGCAACCA AGTCGAAGTG ATAGCCACAC TGAAGGATGG
201 GAGGAAAATC TGCCTGGACC CAGATGCTCC CAGAATCAAG AAAATTGTAC
251 AGAAAAAATT GGCAGGTGAT GAATCTGCTG AT

10 which corresponds to the cDNA sequence and derived amino acid sequence of platelet basic protein. See Wenger et al., *Blood*, 73: 1498-1503, 1989 as well as Walz and Baggiolini, *BBRC* 159: 969-981, 1989; Castor, et al., *BBRC* 163: 1071-1078, 1989.

In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 5) of:

15 Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys
1 5 10 15

Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser
20 25 30

20 Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn Gln Val Glu Val Ile
35 40 45

25 Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro
50 55 60

Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala
65 70 75 80

30 Asp

encoded by the cDNA sequence (SEQ ID NO: 6) of:

1 GGCAAAGAGG AAAGTCTAGA CAGTGACTTG TATGCTGAAC TCCGCTGCAT
51 GTGTATAAAG ACAACCTCTG GAATTCATCC CAAAAACATC CAAAGTTTGG
101 AAGTGATCGG GAAAGGAACC CATTGCAACC AAGTCGAAGT GATAGCCACA
35 151 CTGAAGGATG GGAGGAAAAT CTGCCTGGAC CCAGATGCTC CCAGAATCAA
201 GAAAATTGTA CAGAAAAAAT TGGCAGGTGA TGAATCTGCT GAT

which corresponds to the cDNA sequence and derived amino acid sequence β -thromboglobulin (β TG). See Wenger et al., *Blood*, 73: 1498-1503, 1989.

In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 7) of:

40

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Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys
 1 5 10 15
 Asn Ile Gln Ser Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn Gln
 5 20 25 30
 Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp
 35 40 45
 10 Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly
 50 55 60
 Asp Glu Ser Ala Asp
 65
 15

encoded by the cDNA sequence (SEQ ID NO: 8) of:

1 GAACTCCGCT GCATGTGTAT AAAGACAACC TCTGGAATTC ATCCCCAAAA
 51 CATCCAAAGT TTGGAAGTGA TCGGGAAAGG AACCCATTGC AACCAAGTCG
 101 AAGTGATAGC CACACTGAAG GATGGGAGGA AAATCTGCCT GGACCCAGAT
 20 151 GCTCCCAGAA TCAAGAAAAT TGTACAGAAA AAATTGGCAG GTGATGAATC
 201 TGCTGAT

which corresponds to the cDNA sequence and derived amino acid sequence of neutrophil activating peptide-2.

The foregoing amino acid sequences correspond to the products of a single gene called
 25 platelet basic protein (Walz and Baggiolini, *BBRC* 159: 969-981, 1989; Castor, *et al.*, *BBRC*
 163: 1071-1078, 1989). The complete gene sequence of platelet basic protein is well known.
 See, for example, Wenger *et al.*, *Blood*, 73: 1498-1503, 1989 and *Proc. Natl. Acad. Sci. USA*,
 90, 3660-3664, 1993.

The present invention also provides heparanase having the amino acid sequences of
 30 other members of the CXC chemokine family [including Platelet factor 4 (SEQ. ID NO. 12),
 γ MIP-10 (SEQ. ID NO. 14), *gro*/MGSA (SEQ. ID NO. 16), *gro*- β /MIP-2 α (SEQ. ID NO. 18),
gro- γ MIP-2 β (SEQ. ID NO. 20), Interleukin-8/NAP-1 (SEQ. ID NO. 22) and ENA-78 (SEQ. ID
 NO. 24)] as well as members of the CC chemokine family [including MIP-1 α (SEQ. ID NO.
 26), MIP-1 β (SEQ. ID NO. 28), I-309 (SEQ. ID NO. 23), MCP-1 (SEQ. ID NO. 32), MCP-3
 35 (SEQ. ID NO. 34), RANTES (SEQ. ID NO. 36), fic (SEQ. ID NO. 38) and MCP-2 (SEQ. ID
 NO. 40)]; purified to apparent homogeneity, prepared in the presence of reducing conditions,
 and activated by treatment with transglutaminase. Suitable transglutaminases that may be used
 for this purpose include Activated Factor XIIIa, guinea pig liver transglutaminase, epidermal
 transglutaminase, keratinocyte transglutaminase, and tissue transglutaminase.

40 In another aspect, the present invention provides a heparanase having the amino acid

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sequence (SEQ ID NO: 12) of:

Met Ser Ser Ala Ala Gly Phe Cys Ala Ser Arg Pro Gly Leu Leu Phe Leu Gly Leu Leu
 Leu Leu Pro Leu Val Val Ala Phe Ala Ser Ala Glu Ala Glu Glu Asp Gly Asp Leu Gln
 Cys Leu Cys Val Lys Thr Thr Ser Gln Val Arg Pro Arg His Ile Thr Ser Leu Glu Val
 5 Ile Lys Ala Gly Pro His Cys Pro Thr Ala Gln Leu Ile Ala Thr Leu Lys Asn Gly Arg
 Lys Ile Cys Leu Asp Leu Gln Ala Pro Leu Tyr Lys Lys Ile Ile Lys Lys Leu Leu Glu
 Ser

encoded by the cDNA sequence (SEQ ID NO: 13) of:

1 CCGCAGCATG AGCTCCGCAG CCGGGTTCTG CGCCTCACGC CCCGGGCTGC
 10 51 TGTTCTTGGG GTTGCTGCTC CTGCCACTTG TGGTCGCCTT CGCCAGCGCT
 101 GAAGCTGAAG AAGATGGGGA CCTGCAGTGC CTGTGTGTGA AGACCACCTC
 151 CCAGGTCCGT CCCAGGCACA TCACCAGCCT GGAGGTGATC AAGGCCGGAC
 201 CCCACTGCCC CACTGCCCAA CTGATAGCCA CGCTGAAGAA TGGAAGGAAA
 251 ATTTGCTTGG ACCTGCAAGC CCCGCTGTAC AAGAAAATAA TTAAGAAACT
 15 301 TTTGGAGAGT TAGCTACTAG CTGCCTACGT GTGTGCATTT GCTATATAGC
 351 ATACTTCTTT TTTCCAGTTT CAATCTAACT GTGAAAGAAA CTTCTGATAT
 401 TTGTGTTATC CTTATGATTT TAAATAAACA AAATAAATC

which corresponds to the cDNA sequence and derived amino acid sequence of platelet factor 4.
 See Poncz et al., *Blood* 69, 219-223 (1987).

20 In another aspect, the present invention provides a heparanase having the amino acid
 sequence (SEQ ID NO: 14) of:

Met Asn Gln Thr Ala Ile Leu Ile Cys Cys Leu Ile Phe Leu Thr Leu Ser Gly Ile Gln Gly
 Val Pro Leu Ser Arg Thr Val Arg Cys Thr Cys Ile Ser Ile Ser Asn Gln Pro Val Asn Pro
 Val Asn Pro Arg Ser Leu Glu Lys Leu Glu Ile Ile Pro Ala Ser Gln Phe Cys Pro Arg
 25 Val Glu Ile Ile Ala Thr Met Lys Lys Lys Gly Glu Lys Arg Cys Leu Asn Pro Glu Ser
 Lys Ala Ile Lys Asn Leu Leu Lys Ala Val Ser Lys Glu Met Ser Lys Arg Ser Pro

encoded by the cDNA sequence (SEQ ID NO: 15) of:

1 GAGACATTCC TCAATTGCTT AGACATATTC TGAGCCTACA GCAGAGGAAC
 51 CTCCAGTCTC AGCACCATGA ATCAAAGTGC GATTCTGATT TGCTGCCTTA
 30 101 TCTTTCTGAC TCTAAGTGGC ATTCAAGGAG TACCTCTCTC TAGAACCGTA
 151 CGCTGTACCT GCATCAGCAT TAGTAATCAA CCTGTTAATC CAAGGTCTTT
 201 AGAAAAACTT GAAATTATTC CTGCAAGCCA ATTTTGTCCA CGTGTGAGA
 251 TCATTGCTAC AATGAAAAAG AAGGGTGAGA AGAGATGTCT GAATCCAGAA
 301 TCGAAGGCCA TCAAGAATTT ACTGAAAGCA GTTAGCAAGG AAATGTCTAA
 35 351 AAGATCTCCT TAAAACCAGA GGGGAGCAAA ATCGATGCAG TGCTTCCAAG
 401 GATGGACCAC ACAGAGGCTG CCTCTCCCAT CACTCCCTA CATGGAGTAT

451 ATGTCAAGCC ATAATTGTTC TTAGTTTGCA GTTACACTAA AAGGTGACCA
501 ATGATGGTCA CCAAATCAGC TGCTACTACT CCTGTAGGAA GGTTAATGTT
551 CATCATCCTA AGCTATTCAG TAATAACTCT ACCCTGGCAC TATAATGTAA
601 GCTCTACTGA GGTGCTATGT TCTTAGTGGA TGTCTGACC CTGCTTCAAA

5 which corresponds to the cDNA sequence and derived amino acid sequence γ IP-10. See Luster et al., Nature 315, 672-676 (1985).

In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 16) of:

Met Ala Arg Ala Ala Leu Ser Ala Ala Pro Ser Asn Pro Arg Leu Leu Arg Val Ala Leu
10 Leu Leu Leu Leu Val Ala Ala Gly Arg Arg Ala Ala Gly Ala Ser Val Ala Thr Glu
Leu Arg Cys Gln Cys Leu Gln Thr Leu Gln Gly Ile His Pro Lys Asn Ile Gln Ser Val
Asn Val Lys Ser Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys Asn
Gly Arg Lys Ala Cys Leu Asn Pro Ala Ser Pro Ile Val Lys Lys Ile Ile Glu Lys Met
Leu Asn Ser Asp Lys Ser Asn

15 encoded by the cDNA sequence (SEQ ID NO: 17) of:

1 CTCGCCAGCT CTTCCGCTCC TCTCACAGCC GCCAGACCCG CCTGCTGAGC
51 CCCATGGCCC GCGCTGCTCT CTCCGCCGCC CCCAGCAATC CCCGGCTCCT
101 GCGAGTGGCA CTGCTGCTCC TGCTCCTGGT AGCCGCTGGC CGGCGCGCAG
151 CAGGAGCGTC CGTGGCCACT GAACTGCGCT GCCAGTGCTT GCAGACCCTG
20 201 CAGGGAATTC ACCCCAAGAA CATCCAAAGT GTGAACGTGA AGTCCCCCGG
251 ACCCCACTGC GCCCAAACCG AAGTCATAGC CAACTCAAG AATGGGCGGA
301 AAGCTTGCCT CAATCCTGCA TCCCCCATAG TTAAGAAAAT CATCGAAAAG
351 ATGCTGAACA GTGACAAATC CAACTGACCA GAAGGGAGGA GGAAGCTCAC
401 TGGTGGCTGT TCCTGAAGGA GGCCCTGCCC TTATAGGAAC AGAAGAGGAA
25 451 AGAGAGACAC AGCTGCAGAG GCCACCTGGA TTGTGCCTAA TGTGTTTGAG
501 CATCGCTTAG GAGAAGTCTT CTATTTATTT ATTTATTCAT TAGTTTTGAA
551 GATTCTATGT TAATATTTTA GGTGTAAAAT AATTAAGGGT ATGATTAAC
601 CTACCTGCAC ACTGTCCTAT TATATTCATT CTTTTTGAAA TGTCAACCCC
651 AAGTTAGTTC AATCTGGATT CATATTTAAT TTGAAGGTAG AATGTTTTCA
30 701 AATGTTCTCC AGTCATTATG TTAATATTTT TGAGGAGCCT GCAACATGCC
751 AGCCACTGTG ATAGAGGCTG GCGGATCCAA GCAAATGGCC AATGAGATCA
801 TTGTGAAGGC AGGGGAATGT ATGTGCACAT CTGTTTTGTA ACTGTTTAGA
851 TGAATGTCAG TTGTTATTTA TTGAAATGAT TTCACAGTGT GTGGTCAACA
901 TTTCTCATGT TGAACTTTA AGAACTAAAA TGTCTAAAT ATCCCTTGGA
35 951 CATTTTATGT CTTTCTTGTA AGGCATACTG CCTGTGTTAA TGGTAGTTTT
1001 ACAGTGTTC TGGCTTAGAA CAAAGGGGCT TAATTATTGA TGTTCGGA

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which corresponds to the cDNA sequence and derived amino acid sequence of *gro*/MGSA (melanoma growth stimulatory activity). See Anisowicz et al., Proc. Natl. Acad. Sci. U.S.A. 84, 7188-7192 (1987).

In another aspect, the present invention provides a heparanase having the amino acid
5 sequence (SEQ ID NO: 18) of:

Met Ala Arg Ala Thr Leu Ser Ala Ala Pro Ser Asn Pro Arg Leu Leu Arg Val Ala Leu
Leu Leu Leu Leu Leu Val Ala Ala Ser Arg Arg Ala Ala Gly Ala Pro Lys Ala Thr Glu
Lys Arg Cys Gln Cys Lys Gln Thr Leu Gln Gly Ile His Leu Lys Asn Ile Gln Ser Val
Lys Val Lys Ser Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys Asn
10 Gly Gln Lys Ala Cys Leu Asn Pro Ala Ser Pro Met Val Lys Lys Ile Ile Glu Lys Met
Leu Lys

encoded by the cDNA sequence (SEQ ID NO: 19) of:

1 CTCTCCTCCT CGCACAGCCG CTCGAACCGC CTGCTGAGCC CCATGGCCCCG
51 CGCCACGCTC TCCGCCGCC CCAGCAATCC CCGGCTCCTG CGGGTGGCGC
15 101 TGCTGCTCCT GTCCTGGTG GCCGCCAGCC GGCGCGCAGC AGGAGCGCCC
151 CTGGCCACTG AACTGCGCTG CCAGTGCTTG CAGACCCTGC AGGGAATTCA
201 CCTCAAGAAC ATCCAAAGTG TGAAGGTGAA GTCCCCCGGA CCCCCTGCG
251 CCCAAACCGA AGTCATAGCC AACTCAAGA ATGGGCAGAA AGCTTGCTC
301 AACCCCGCAT CGCCCATGGT TAAGAAAATC ATCGAAAAGA TGCTGAAAAA
20 351 TGGCAAATCC AACTGACCAG AAGGAAGGAG GAAGCTTATT GGTGGCTGTT
401 CCTGAAGGAG GCCCTGCCCT TACAGGAACA GAAGAGGAAA GAGAGACACA
451 GCTGCAGAGG CCACCTGGAT TGCGCTAAT GTGTTTGAGC ATCACTTAGG
501 AGAAGTCTTC TATTTATTTA TTTATTTATT TATTTGTTTG TTTAGAAGA
551 TTCTATGTTA ATATTTTATG TGTAATAA GGTTATGATT GAATCTACTT
25 601 GCACACTCTC CCATTATATT TATTGTTTAT TTAGGTCAA ACCCAAGTTA
651 GTTCAATCCT GATTCATATT TAATTTGAAG ATAGAAGGTT TGCAGATATT
701 CTCTAGTCAT TTGTTAATAT TTCTTCGTGA TGACATATCA CATGTCAGCC
751 ACTGTGATAG AGGCTGAGGA ATCCAAGAAA ATGGCCAGTG AGATCAATGT
801 GACGGCAGGG AAATGTATGT GTGTCTATTT TGTAAGTGA AAGATGAATG
30 851 TCAGTTGTTA TTTATTGAAA TGATTTTACA GTGTGTGGTC AACATTTCTC
901 ATGTTGAAGC TTTAAGAACT AAAATGTTCT AAATATCCCT TGGACATTTT
951 ATGTCTTTCT TGTAAGGCAT ACTGCCTTGT TTAATGTAA TTATGCAGTG
1001 TTTCCCTCTG TGTTAGAGCA GAGAGGTTTC GATATTTATT GATGTTTTCA
1051 CAAAGAACAG GAAAATAAAA TATTTAAAAA T

35 which corresponds to the cDNA sequence and derived amino acid sequence *gro*- β /MIP-2 α (macrophage inflammatory protein 2- α). See Tekamp-Olson et al., J. Exp. Med. 172, 911-919

(1990).

In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 20) of:

Met Ala His Ala Thr Leu Ser Ala Ala Pro Ser Asn Pro Arg Leu Leu Arg Val Ala Leu
 5 Leu Leu Leu Leu Val Ala Ala Ser Arg Arg Ala Ala Gly Ala Ser Val Val Thr Glu
 Leu Arg Cys Gln Cys Leu Gln Thr Leu Gln Gly Ile His Leu Lys Asn Ile Gln Ser Val
 Asn Val Arg Ser Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys Asn
 Gly Lys Lys Ala Cys Leu Asn Pro Ala Ser Pro Met Val Gln Lys Ile Ile Glu Lys Ile Leu
 Asn Lys Gly Ser Thr Asn

10 encoded by the cDNA sequence (SEQ ID NO: 21) of:

1 CTCGCACAGC TTCCCGACGC GTCTGCTGAG CCCCATGGCC CACGCCACGC
 51 TCTCCGCCGC CCCAGCAAT CCCCGGCTCC TGCGGGTGGC GCTGCTGCTC
 101 CTGCTCCTGG TGGCCGCCAG CCGGCGCGCA GCAGGAGCGT CCGTGGTCAC
 151 TGAAGTGGCG TGCCAGTGCT TGCAGACACT GCAGGGAATT CACCTCAAGA
 15 201 ACATCCAAAG TGTGAATGTA AGGTCCCCCG GACCCCACTG CGCCCAAACC
 251 GAAGTCATAG CCACACTCAA GAATGGGAAG AAAGCTTGTC TCAACCCCGC
 301 ATCCCCCATG GTTCAGAAAA TCATCGAAAA GATACTGAAC AAGGGGAGCA
 351 CCAACTGACA GGAGAGAAGT AAGAAGCTTA TCAGCGTATC ATTGACACTT
 401 CCTGCAGGGT GGTCCCTGCC CTTACCAGAG CTGAAAATGA AAAAGAGAAC
 20 451 AGCAGCTTTC TAGGGACAGC TGGAAAGGAC TTAATGTGTT TGAATATTTT
 501 TTACGAGGGT TCTACTTATT TATGTATTTA TTTTGAAAG CTGTATTTT
 551 AATATTTTAC ATGCTGTTAT TAAAGATGT GAGTGTGTTT CATCAAACAT
 601 AGCTCAGTCC TGATTATTTA ATTGGAATAT GATGGGTTTT AAATGTGTCA
 651 TTAAACTAAT ATTTAGTGGG AGACCATAAT GTGTCAGCCA CCTTGATAAA
 25 701 TGACAGGGTG GGGAAGTGA GGGTGGGGGG ATTGAAATGC AAGCAATTAG
 751 TGGATCACTG TTAGGGTAAG GGAATGTATG TACACATCTA TTTTTATAC
 801 TTTTTTTTA AAAAAAGAAT GTCAGTTGTT ATTTATTCAA ATTATCTCAC
 851 ATTATGTGTT CAACATTTTT ATGCTGAAGT TTCCCTTAGA CATTTTATGT
 901 CTGCTTGTA GGGCATAATG CCTTGTTTAA TGTCCATTCT GCAGCGTTTC
 30 951 TCTTTCCCTT GGAAAAGAGA ATTTATCATT ACTGTTAC

which corresponds to the cDNA sequence and derived amino acid sequence *gro-γ*MIP-2β (macrophage inflammatory protein 2-β). See Tekamp-Olson et al., J. Exp. Med. 172, 911-919 (1990).

In another aspect, the present invention provides a heparanase having the amino acid
 35 sequence (SEQ ID NO: 22) of:

Met Thr Ser Lys Leu Ala Val Ala Leu Leu Ala Ala Phe Leu Ile Ser Ala Ala Leu Cys

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Glu Gly Ala Val Leu Pro Arg Ser Ala Lys Glu Leu Arg Cys Gln Cys Ile Lys Thr Tyr
 Ser Lys Pro Phe His Pro Lys Phe Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro His Cys
 Ala Asn Thr Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu Leu Cys Leu Asp Pro Lys
 Glu Asn Trp Val Gln Arg Val Val Glu Lys Phe Leu Lys Arg Ala Glu

5 encoded by the cDNA sequence (SEQ ID NO: 23) of:

1 ATGACTTCCA AGCTGGCCGT GGCTCTCTTG GCAGCCTTCC TGATTTCCTGC
 51 AGCTCTGTGT GAAGGTGCAG TTTTGCCAAG GAGTGCTAAA GAACTTAGAT
 101 GTCAGTGCAT AAAGACATAC TCCAAACCTT TCCACCCCAA ATTTATCAAA
 151 GAACTGAGAG TGATTGAGAG TGGACCACAC TGCGCCAACA CAGAAATTAT
 10 201 TGTAAGCTT TCTGATGGAA GAGAGCTCTG TCTGGACCCC AAGGAAAAC
 251 GGGTGCAGAG GGTTGTGGAG AAGTTTTTGA AGAGGGCTGA G

which corresponds to the cDNA sequence and derived amino acid sequence Interleukin-8/NAP-1 (neutrophil activating protein-1). See Kunser et al., *Kidney Int.* 39, 1240-1248 (1991).

In another aspect, the present invention provides a heparanase having the amino acid
 15 sequence (SEQ ID NO: 24) of:

Ala Gly Pro Ala Ala Val Leu Arg Glu Lys Arg Cys Val Cys Leu Gln Thr Thr Gln
 Gly Val His Pro Lys Met Ile Ser Asn Leu Gln Val Phe Ala Ile Gly Pro Gln Cys Ser
 Lys Val Glu Val Val Ala Ser Leu Lys Asn Gly Lys Glu Ile Cys Leu Asp Pro Glu Ala
 Pro Phe Leu Lys Lys Val Ile Gln Lys Ile Leu Asp Gly Gly Asn Lys Glu Asn

20 encoded by the cDNA sequence (SEQ ID NO: 25) of:

1 GTGTTGCGGG AACTGCGGTG CGTGTGTTTA CAGACCACGC AGGGAGTTCA
 51 TCCCAAATG ATCAGTAATC TGCAAGTGTT CGCCATAGGC CCACAGTGCT
 101 CCAAGGTGGA AGTGGTAGCC TCCCTGAAGA ACGGGAAGGA AATTTGTCTT
 151 GATCCAGAAG CCCCTTTTCT AAAGAAAGTC ATCCAGAAAA TCCTCGACGG
 25 201 CGGCAACAAA GAAAAC

which corresponds to the cDNA sequence and derived amino acid sequence of a novel inflammatory peptide (ENA-78) with homology to interleukin 8. See Walz et al., *J. Exp. Med.* 174, 1355-1362 (1991).

In another aspect, the present invention provides a heparanase having the amino acid
 30 sequence (SEQ ID NO: 26) of:

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala Leu Cys Asn Gln
 Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr Ala Cys Cys Phe Ser Tyr Thr Ser
 Arg Gln Ile Pro Gln Asn Phe Ile Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys
 Pro Ser Val Ile Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
 35 Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala

encoded by the cDNA sequence (SEQ ID NO: 27) of:

1 GAATTCAAGG CCTGTCCTGG TTTGGTCCCA ATTTACCTTT ATCATCCATA
51 TTCACCCCCA CTGCTCTGCA GCTCCACTGA AGCACCCCCT CTTTCCTCTG
101 AGCCACAATG TCACACCCAG GACTCTGCCT CAGCTGGGCC TCCACTGCCC
151 ACCCATCTAT AGATGCCTAA ATCCCGGGCA GTTATCCAGA CACAACTAAA
5 201 GTTCCATCCC TTCCATGAAG CCTTCCCCAA CCTCTGGTG GAAGGTCACT
251 TCTTCCTCAT GGGGTTCTGA GCTTTCATTT CTTTTTCTAO TAAGAGTTTT
301 ACAATTACCT GTTCATACAC TCTACCTGCC CCCATGAGAC CAGGGGCATC
351 TCAGAAACAA AGATCATTA AACCACCTAA ATCTATTTCT CATTATAAAA
401 TGAGATATGC TGATTGATTG CAAAATAATA AAATAACAAA GTATGGAAAA
10 451 GAAAAAAAAA AGCATATAAT CTGGCTGAGA AGGTAGAGAC CCTTCCACAC
501 CACTGAAATT ATGTGTTGAA AAGAATAAGG AAAAACTGC TTCAGTTTGG
551 CATTATTTAT GTAAGTATAG TATAGGATCC TTAAAATGGT TCAAAGAAAT
601 GGGAAATCAA GACTTCATTT TGGCAAAGCC ATTGAACAGA AACTGTAGCA
651 TATTTATCAG TAATTTCTTT CAGATTAAAC AACTGACAAC AACCCACTTT
15 701 TCAACCAGTG ATGTTGGAAA TGTTTTAAAA CAAAATTAGT TCATAAATTT
751 GTGGGTTGAC CAAGAAGGTA ATAAAGTCTC ACTAAATAAA ATGAGGAAAA
801 TTCAGAAAAA GAAAAAATA AGAAAATAAA TCACCCATGG ATCTAAGCAC
851 TATTCATTCT TTAAGGCATG TATTTCCAAG CCTTTTAATT TTTTCATGCC
901 TAGAGTTGGC ATGGCATATA TATATCTTTA TACAATTCTT CAAATTTTAT
20 951 AGAATTTGTA TAATGTTTTA TCTTGCTTTT TTTTAAACCA CTGATGTTAT
1001 AAGCATATTT ATGCCACTTC ATTCACGTTA GAGACTTAAT AATAAAGGAT
1051 CTTGTGGATA ATTTATCATT CCTGATAGA GAAAAATTTA GCTTTGCTTA
1101 TTTTAGAGTT ATAAATGATG CTGGGTCAGG TATCTTTATG TTTGAAGATG
1151 GCTCCATATT TGGGTTGTTT CCACAGAACT CTTTCCAGAA ATGCTTTTTT
25 1201 TAGGTAAATG GCTACACATA TTTCTAGGCA CCTGACATAC TGACACCCAC
1251 CTCTAAAGTA TTTTATGAT CCACAACCTAG CGTTTAAACAC AGCGCCCCAG
1301 TCACTCCGAG ACTAATAAAT AGACAAATGA CTGAAACGTG ACCTCATGCT
1351 TTCTATTCCT CCAGCTTTCA TTGAGTTCCT TTCCTCTGGG AGGACTGGGG
1401 GTTGTCTAGC CCTCCACAGC ATCAGCCCAT TGACCCTATC CTTGTGGTTA
30 1451 TAGCAGCTGA GGAAGCAGAA TTCTAGCTCT GTGGGAAGGA ATGGGGCTGG
1501 AGAGTTCATG CATAGACCAA TTCCTTTTTT TTTTTTTTTT TGAGATGGAG
1551 TTTCACTTTT GTTGCCCAGG CTGGAGTGCA ATGGCATGAT CTCAGCTCAC
1601 CACAGCCCCC ACCTCCTGGG TTCAAGCGAT TCTCCTGCCC TCAGCCTCCC
1651 GAGTAGCTGG GATTACAGGC ATGTGCCACC ACGCCTGACT ACTTTTGTAT
35 1701 TTTTAGTAGA GATGGAGTTT CTCTTCTTG GTCAGGTTGG TCTCAAATC
1751 CTGACCTCAG GTGATCTGCA GCCTCGGCCT CCAAAGTGTT GGGATTACAG

1801 GTGTGAGCGA CCATGCCTGG CTGCATAGAC CAGTTCTTAT GAGAAGGGAT
1851 CAACTAAGAA TAGCCTTGGG TTGACACACA CCCCTCTTCA CACTCACAGG
1901 AGAAACCCCA TGAAGCTAGA ACCAGTCATG AGTTGAGAGC TGAGAGTTAG
1951 AGAGTAGCTC AGAGATGCTA TTCTTGGATA TCCTGAGCCC CTGTGGTCAC
5 2001 CAGGGACCCCT GAGTTGTGCA ACACTCAGCA TGACAGCATC ACTACACTTA
2051 AAAATTTCCC TCCTCACCCC CAGATTCCAT TTCCCCATCC GCCAGGGCTG
2101 CCTATAAAGA GGAGAGATGG CTTCAGACAT CAGAAGGACG CAGGCAGCAA
2151 AGAGTAGTCA GTCCCTTCTT GGCTCTGCTG ACACTCGAGC CCACATTCCA
2201 TCACCTGCTC CCAATCATGC AGGTCTCCAC TGCTGCCCTT GCCGTCCTCC
10 2251 TCTGCACCAT GGCTCTCTGC AACCAGGTCC TCTCTGCACC ACGTGAGTCC
2301 ATGTTGTTGT TGTGGGTATC ACCACTCTCT GGCCATGGTT AGACCACATC
2351 AGTCTTTTTT TGTGGCGTGA GAGGCCCCGA AGAGAAAAGA AGGAAGTTCT
2401 TAAAGCGCTG CCAAACACCT TGGTCTTTTT CTCACAAC TTTATTTTA
2451 TCTCTAGAAG GGGTCTTAGC CCTCCTAGTC TCCAGGTATG AGAATCTAGG
15 2501 CAGGGGCAGG GGAGTTACAG TCCCTTGTAC AGATAGAAAA ACAGGGTTCA
2551 AAACGAATCA GTTTGCAAGA GGCAGAATCC AGGGCTGCTT ACTTCCCAGT
2601 GGGGTCTGTT CTTCACTCTC CAGCTACCC TAGTCTCCCA GGAGCCCTGT
2651 CCCTTGATG TCTTATGAGA GATGTCCAGG GCTTCTCTTG GGCTGGGGTA
2701 TGA CTCTTG AACCGACAAA ATTCCATGAA GAGAGCTAAG AGAACAGTCC
20 2751 ATTCAGGTAT CTGGATCACA TAGAGAAACA GAGAACCAC TATGAAGAGT
2801 CAAGGGGAAA GAGGAATATA GACAGAAACA AAGAGACATT TCTCTGCAAA
2851 ACCCCCCAAA TGCCTTGCAG TCACTTGGTC TGAGCAAGCC TGCCCTCCTC
2901 AACCCTCAG GGATCAGAAG CTGCCTGGCC TTTCTTCTG AGCTGTGACT
2951 TGGGCTTATT CTCTCCTTC TCCGCAGTTG CTGCTGACAC GCCGACCGCC
25 3001 TGCTGCTTCA GCTACACCTC CCGACAGATT CCACAGAATT TCATAGCTGA
3051 CTACTTTGAG ACGAGCAGCC AGTGCTCAA GCCCAGTGTC ATGTAAGTGC
3101 CAGTCTTCCT GCTCACCTCT AGGGAGGTAG GGAGTGTGAG GGTGGGGGCA
3151 GAAACAGGCC AGAAGGCCAT CTGGAAAGG CCCAGCCTTC AGGAGCCTAT
3201 CGGGGATACA GGACGCAGGG CACTGAGGTG TGACCTGACT TGGGGCTGGA
30 3251 GTGAGGTGGG TGTTACAGAG TCAGGAAGGG CTGCCCCAGG CCAGAGGAAA
3301 GGGACAGGAA GAAGGAGGCA GCAGGACACT CTGAGGGCCC CCTTGCCTGG
3351 AGTCACTGAG AGAAGCTCTC TAGACGGAGA TAGGCAGGGG GCCCCTGAGA
3401 GAGGAGCAGG CCTTGAGCTG CCCAGGACAG AGAGCAGGAT GTCAGGGCCA
3451 TGGTGGGCCC AGGATTCCCC GGCTGGATTG CCCAGTGCTT AACTCTTCCT
35 3501 CCCTTCTCCA CAGCTTCCTA ACCAAGAGAG GCCGGCAGGT CTGTGCTGAC
3551 CCCAGTGAGG AGTGGGTCCA GAAATACGTC AGTGACCTGG AGCTGAGTGC

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3601 CTGAGGGGTC CAGAAGCTTC GAGGCCAGC GACCTCAGTG GGCCCACTGG
 3651 GGAGGAGCAG GAGCCTGAGC CTTGGGAACA TGCGTGTGAC CTCCACAGCT
 3701 ACCTCTTCTA TGGACTGGTT ATTGCCAAAC AGCCACACTG TGGGACTCTT
 3751 CTTAACTTAA ATTTTAATTT ATTTATACTA TTTAGTTTTT ATAATTTATT
 5 3801 TTTGATTTC AAGTGTGTTT GTGATTGTTT GCTCTGAGAG TTCCCCCTGT
 3851 CCCCTCCACC TTCCCTCACA GTGTGTCTGG TGACAACCGA GTGGCTGTCA
 3901 TCGGCCTGTG TAGGCAGTCA TGGCACCAAA GCCACCAGAC TGACAAATGT
 3951 GTATCAGATG CTTTGTGTTCA GGGCTGTGAT CGGCCTGGG AAATAATAAA
 4001 GATGTTCTTT TAAACGGTAA ACCAGTATTG AGTTTGGTTT TGTTTTTCTG
 10 4051 GCAAATCAAA ATCACTGGTT AAGAGGAATC ATAGGCAAAG ATTAGGAAGA
 4101 GGTGAAATGG AGGGAAATTG GGAGAGATGG GGAGCGCTGC GACAGAGTTA
 4151 TCCACTTGAC AAAATTTCTGG AACATTGAAA CTACGAATAT GTTATAACTG
 4201 AAATCGTAAT ATGCACGCTC TAGGAGAATT AACTACTTGA ATGGCCACC
 4251 TTAAGCAGAG TATTCTGTAG GGCATATTCA TGATGAATCA AGCTCTTAAT
 15 4301 AGCAATTATT TACATTGTTG AGGCTTACTC CTCCTACTGA GTGCTTTTTA
 4351 TACATTGTTT ATTTAATCTT ACCAATGCAA TAGTACAGCT TAGGTACTAT
 4401 TAATACCTCC ACTTGACAGA AAAGTAACCC AGGGCTCAGA AAGGTTAGAC
 4451 AACTTGGCTG AGGTTACACA GCACGTAAAC GGTCAATTGT GTTCCAAAAC
 4501 TGGACTTTTA TTGAACTACA GACTATGCTG TTAACCATTG ACCAAGTTAT
 20 4551 TTCCCAAAGT ATGACCCGCC TATACTCAAA TCTTACCCCA TTCTTTAACA
 4601 GATGATACTT TATCCATTGC AACCCTTCC TGTCAGGATT CTGAGTTGAC
 4651 ATAGAGTGTT TCAGCAGTGA TTATTTAAGC CAATTACATC AGGATCTTTA
 4701 GGTGTAGACC TGGGAACTGA TATTTTATC AAGCTCATGA GGTGTTCCAT
 4751 AGCATGTAA TGAAGTGAAG CCACTGTCAA TAGAATTC

25 which corresponds to the cDNA sequence and derived amino acid sequence MIP-1 α
 (macrophage inflammatory protein 1- α). See Blum et al., DNA Cell Biol. 9, 589-602 (1990).

In another aspect, the present invention provides a heparanase having the amino acid
 sequence (SEQ ID NO: 28) of:

30 Met Lys Leu Cys Val Thr Val Leu Ser Leu Leu Met Leu Val Ala Ala Phe Cys Ser Pro
 Ala Leu Ser Ala Pro Met Gly Ser Asp Pro Pro Thr Ala Cys Cys Phe Ser Tyr Thr Ala
 Arg Lys Leu Pro Arg Asn Phe Val Val Asp Tyr Tyr Glu Thr Ser Ser Leu Cys Ser Gln
 Pro Ala Val Val Phe Gln Thr Lys Arg Ser Lys Gln Val Cys Ala Asp Pro Ser Glu Ser
 Trp Val Gln Glu Tyr Val Tyr Asp Leu Glu Leu Asn

encoded by the cDNA sequence (SEQ ID NO: 29) of:

35 1 TTCCCCCCCC CCCCCCCCCC CCCC GCCCGA GCACAGGACA CAGCTGGGTT
 51 CTGAAGCTTC TGAGTTCTGC AGCCTCACCT CTGAGAAAAC CTCTTTTCCA

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101 CCAATACCAT GAAGCTCTGC GTGACTGTCC TGTCTCTCCT CATGCTAGTA
 151 GCTGCCTTCT GCTCTCCAGC GCTCTCAGCA CCAATGGGCT CAGACCCTCC
 201 CACCGCCTGC TGCTTTTCTT ACACCGCGAG GAAGCTTCCT CGCAACTTTG
 251 TGGTAGATTA CTATGAGACC AGCAGCCTCT GCTCCCAGCC AGCTGTGGTA
 5 301 TTCCAAACCA AAAGAAGCAA GCAAGTCTGT GCTGATCCCA GTGAATCCTG
 351 GGTCCAGGAG TACGTGTATG ACCTGGAAC TGAAGTGTGCT GCTCAGAGAC
 401 AGGAAGTCTT CAGGGAAGGT CACCTGAGCC CGGATGCTTC TCCATGAGAC
 451 ACATCTCCTC CATACTCAGG ACTCCTCTCC GCAGTTCCTG TCCCTTCTCT
 501 TAATTTAATC TTTTTTATGT GCCGTGTTAT TGTATTAGGT GTCATTTCCA
 10 551 TTATTTATAT TAGTTTAGCC AAAGGATAAG TGTCCATATG GGATGGTCCA
 601 CTGTCACTGT TTCTCTGCTG TTGCAAATAC ATGGATAACA CATTGATTG
 651 TGTGTGTTTT CCATAATAAA ACTTTAAAAT AAAATGCAGA CAGTTA

which corresponds to the cDNA sequence and derived amino acid sequence MIP-1 β

(macrophage inflammatory protein 1- β). See Lipes et al., Proc. Natl. Acad. Sci. U.S.A. 85,

15 9704-9708 (1988).

In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 30) of:

Met Gln Ile Ile Thr Thr Ala Leu Val Cys Leu Leu Leu Ala Gly Met Trp Pro Glu Asp
 Val Asp Ser Lys Ser Met Gln Val Pro Phe Ser Arg Cys Cys Phe Ser Phe Ala Glu Gln
 20 Glu Ile Pro Leu Arg Ala Ile Leu Cys Tyr Arg Asn Thr Ser Ser Ile Cys Ser Asn Glu
 Gly Leu Ile Phe Lys Leu Lys Arg Gly Lys Glu Ala Cys Ala Leu Asp Thr Val Gly Trp
 Val Gln Arg His Arg Lys Met Leu Arg His Cys Pro Ser Lys Arg Lys

encoded by the cDNA sequence (SEQ ID NO: 31) of:

1 ACCAGGCTCA TCAAAGCTGC TCCAGGAAGG CCCAAGCCAG ACCAGAAGAC
 25 51 ATGCAGATCA TCACCACAGC CCTGGTGTGC TTGCTGCTAG CTGGGATGTG
 101 GCCGGAAGAT GTGGACAGCA AGAGCATGCA GGTACCCCTC TCCAGATGTT
 151 GCTTCTCATT TGCGGAGCAA GAGATTCCCC TGAGGGCAAT CCTGTGTTAC
 201 AGAAATACCA GCTCCATCTG CTCCAATGAG GGCTTAATAT TCAAGCTGAA
 251 GAGAGGCAAA GAGGCCTGCG CCTTGGACAC AGTTGGATGG GTTCAGAGGC
 30 301 ACAGAAAAAT GCTGAGGCAC TGCCCGTCAA AAAGAAAATG AGCAGATTTT
 351 TTTCCATTGT GGGCTCTGGA AACCACATGG CTTACCTGT CCCCAGAACT
 401 ACCAGCCCTA CACCATTCTT TCTGCCCTGC TTTTGCTAGG TCACAGAGGA
 451 TCTGCTTGGT CTTGATAAGC TATGTTGTTG CACTTTAAAC ATTTAAATTA
 501 TACAATCATC AACCCCCAAC

35 which corresponds to the cDNA sequence and derived amino acid sequence human secreted
 protein (I-309). See Miller et al., J. Immunol. 143, 2907-2916 (1989).

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In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 32) of:

Met Lys Val Ser Ala Ala Leu Leu Cys Leu Leu Leu Ile Ala Ala Thr Phe Ile Pro Gln
Gly Lys Ala Gln Pro Asp Ala Ile Asn Ala Pro Val Thr Cys Cys Tyr Asn Phe Thr Asn
5 Arg Lys Ile Ser Val Gln Arg Leu Ala Ser Tyr Arg Arg Ile Thr Ser Ser Lys Cys Pro Lys
Glu Ala Val Ile Phe Lys Thr Ile Val Ala Lys Glu Ile Ala Ala Pro Lys Gln Lys Trp
Val Gln Asp Ser Met Asp His Leu Asp Lys Gln Thr Gln Thr Pro Lys Thr

encoded by the cDNA sequence (SEQ ID NO: 33) of:

1 CTAACCCAGA AACATCCAAT TCTCAAACCTG AAGCTCGCAC TCTCGCCTCC
10 51 AGCATGAAAG TCTCTGCCGC CCTTCTGTGC CTGCTGCTCA TAGCAGCCAC
101 CTTCAATCCC CAAGGGCTCG CTCAGCCAGA TGCAATCAAT GCCCCAGTCA
151 CCTGCTGTTA TAACTTCACC AATAGGAAGA TCTCAGTGCA GAGGCTCGCG
201 AGCTATAGAA GAATCACCAG CAGCAAGTGT CCCAAAGAAG CTGTGATCTT
251 CAAGACCATT GTGGCCAAGG AGATCTGTGC TGACCCCAAG CAGAAGTGGG
15 301 TTCAGGATT CATGGACCAC CTGGACAAGC AAACCCAAAC TCCGAAGACT
351 TGAACACTCA CTCCACAACC CAAGAATCTG CAGCTAACTT ATTTTCCCCT
401 AGCTTTCCCC AGACACCCTG TTTTATTTTA TTATAATGAA TTTTGTTTGT
451 TGATGTGAAA CATTATGCCT TAAGTAATGT TAATTCCTAT TTAAGTTATT
501 GATGTTTTAA GTTTATCTTT CATGGTACTA GTGTTTTTTA GATACAGAGA
20 551 CTTGGGGAAA TTGCTTTTCC TCTTGAACCA CAGTTCTACC CCTGGGATGT
601 TTTGAGGGTC TTGCAAGAA TCATTAATAC AAAGAATTTT TTTTAACATT
651 CCAATGCATT GCTAAAATAT TATTGTGGAA ATGAATATTT TGTAACATT
701 ACACCAAATA AATATATTTT TGTAC

which corresponds to the cDNA sequence and derived amino acid sequence monocyte
25 chemoattractant protein 1 (MCP-1). See Yoshimura et al., FEBS Lett. 244, 487-493 (1989).

In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 34) of:

Met Lys Ala Ser Ala Ala Leu Leu Cys Leu Leu Leu Thr Ala Ala Ala Phe Ser Pro Gln
Gly Leu Ala Gln Pro Val Gly Ile Asn Thr Ser Thr Thr Cys Cys Tyr Arg Phe Ile Asn
30 Lys Lys Ile Pro Lys Gln Arg Leu Glu Ser Tyr Arg Arg Thr Thr Ser Ser His Cys Pro
Arg Glu Ala Val Ile Phe Lys Thr Lys Leu Asp Lys Glu Ile Cys Ala Asp Pro Thr Gln
Lys Trp Val Gln Asp Phe Met Lys His Leu Asp Lys Lys Thr Gln Thr Pro Lys Leu

encoded by the cDNA sequence (SEQ ID NO: 35) of:

1 AGCAGAGGGG CTGAGACCAA ACCAGAAACC TCCAATTCTC ATGTGGAAGC
35 51 CCATGCCCTC ACCCTCCAAC ATGAAAGCCT CTGCAGCACT TCTGTGTCTG
101 CTGCTCACAG CAGCTGCTTT CAGCCCCCAG GGGCTTGCTC AGCCAGTTGG

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151 GATTAATACT TCAACTACCT GCTGCTACAG ATTTATCAAT AAGAAAATCC
201 CTAAGCAGAG GCTGGAGAGC TACAGAAGGA CCACCAGTAG CCACTGTCCC
251 CGGGAAGCTG TAATCTTCAA GACCAAAGCTG GACAAGGAGA TCTGTGCTGA
301 CCCCACACAG AAGTGGGTCC AGGACTTTAT GAAGCACCTG GACAAGAAAA
5 351 CCCAAACTCC AAAGCTTTGA ACATTCATGA CTGAACTAAA AACAAGCCAT
401 GACTTGAGAA ACAAATAATT TGTATACCCT GTCCTTTCTC AGAGTGGTTC
451 TGAGATTATT TTAATCTAAT TCTAAGGAAT ATGAGCTTTA TGTAAATAATG
501 TGAATCATGG TTTTCTTAG TAGATTTTAA AAGTTATTAA TATTTTAATT
551 TAATCTTCCA TGGATTTTGG TGGGTTTGA ACATAAAGCC TTGGATGTAT
10 601 ATGTCATCTC AGTGCTGTAA AAAGTGTGGG ATGCTCCTCC CTCTCTACC
651 TCATGGGGGT ATTGTATAAG TCCTTGCAAG AATCAGTGCA AAGATTGCT
701 TTAATTGTTA AGATATGATG TCCCTATGGA AGCATATTGT TATTATATAA
751 TTACATATTT GCATATGTAT GACTCCCAA TTTTCACATA AAATAGATTT
801 TTGTAAAAAA

15 which corresponds to the cDNA sequence and derived amino acid sequence monocyte chemoattractant protein 3 (MCP-3). See: Structural and Functional Identification of Two Human, Tumor-derived Monocyte Chemotactic Proteins (MCP-2 and MCP-3) Belonging to the Chemokine Family. Jo Van Damme, Paul Proost, Jean-Pierre Lenaerts, and Ghislain Opdenakker. J. Exp. Med. 176: 59-65, 1992.

20 In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 36) of:

Met Lys Val Ser Ala Ala Arg Leu Ala Val Ile Leu Ile Ala Thr Ala Leu Cys Ala Pro
Ala Ser Ala Ser Pro Tyr Ser Ser Asp Thr Thr Pro Cys Cys Phe Ala Tyr Ile Ala Arg Pro
Leu Pro Arg Ala His Ile Lys Glu Tyr Phe Tyr Thr Ser Gly Lys Cys Ser Asn Pro Ala
25 Val Val Phe Val Thr Arg Lys Asn Arg Gln Val Cys Ala Asn Pro Glu Lys Lys Trp Val
Arg Glu Tyr Ile Asn Ser Leu Glu Met Ser

encoded by the cDNA sequence (SEQ ID NO: 37) of:

1 CCTCCGACAG CCTCTCCACA GGTACCATGA AGGTCTCCGC GGCACGCCTC
51 GCTGTCATCC TCATTGCTAC TGCCCTCTGC GCTCCTGCAT CTGCCTCCCC
30 101 ATATTCCTCG GACACCACAC CCTGCTGCTT TGCCTACATT GCCCGCCCAC
151 TGCCCCGTGC CCACATCAAG GAGTATTTCT ACACCAGTGG CAAGTGCTCC
201 AAGCCAGCAG TCGTCTTTGT CACCCGAAAG AACCGCCAAG TGTGTGCCAA
251 CCCAGAGAAG AAATGGGTTC GGGAGTACAT CAACTCTTTG GAGATGAGCT
301 AGGATGGAGA GTCCTTGAAC CTGAACTTAC ACAAATTTGC CTGTTTCTGC
35 351 TTGCTCTTGT CTTAGCTTGG GAGGCTTCCC CTCCTATCC TACCCACCC
401 GCTCCTTGAA GGGCCCAGAT TCTGACCACG ACGAGCAGCA GTTACAAAAA

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451 CCTTCCCCAG GCTGGACGTG GTGGCTCAGC CTTGTAATCC CAGCACTTTG
 501 GGAGGCCAAG GTGGGTGGAT CACTTGAGGT CAGGAGTTCG AGACAGCCTG
 551 GCCAACATGA TGAAACCCCA TGTGTACTAA AAATACAAAA AATTAGCCGG
 601 GCGTGGTAGC GGGCGCCTGT AGTCCCAGCT ACTCGGGAGG CTGAGGCAGG
 5 651 AGAATGGCGT GAACCCGGGA GCGGAGCTTG CAGTGAGCCG AGATCGCGCC
 701 ACTGCACTCC AGCCTGGGCG ACAGAGCGAG ACTCCGTCTC AAAAAAAAAA
 751 AAAAAAAAAA AAAAAATACA AAAATTAGCC GCGTGGTGGC CCACGCCTGT
 801 AATCCCAGCT ACTCGGGAGG CTAAGGCAGG AAAATTGTTT GAACCCAGGA
 851 GGTGGAGGCT GCAGTGAGCT GAGATTGTGC CACTTCACTC CAGCCTGGGT
 10 901 GACAAAGTGA GACTCCGTCA CAACAACAAC AACAAAAAGC TTCCCCAACT
 951 AAAGCCTAGA AGAGCTTCTG AGGCGCTGCT TTGTCAAAAG GAAGTCTCTA
 1001 GGTCTGAGC TCTGGCTTTG CTTGGCTTT GCAAGGGCTC TGTGACAAGG
 1051 AAGGAAGTCA GCATGCCTCT AGAGGCAAGG AAGGGAGGAA CACTGCACTC
 1101 TTAAGCTTCC GCCGTCTCAA CCCCTCACAG GAGCTTACTG GCAAACATGA
 15 1151 AAAATCGGGG

which corresponds to the cDNA sequence and derived amino acid sequence Human T cell-specific protein (RANTES). See Schall et al., J. Immunol. 141, 1018-1025 (1988).

In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 38) of:

20 Met Arg Ile Ser Ala Thr Leu Leu Cys Leu Leu Leu Ile Ala Ala Ala Phe Ser Ile Gln Val
 Trp Ala Gln Pro Asp Gly Pro Asn Ala Ser Thr Cys Cys Tyr Val Lys Lys Gln Lys Ile
 Pro Lys Arg Asn Leu Lys Ser Tyr Arg Arg Ile Thr Ser Ser Arg Cys Pro Trp Glu Ala
 Val Ile Phe Lys Thr Lys Lys Gly Met Glu Val Cys Arg Glu Ala His Gln Lys Trp Val
 Glu Glu Ala Ile Ala Tyr Leu Asp Met Lys Thr Pro Thr Pro Lys Pro

25 encoded by the cDNA sequence (SEQ ID NO: 39) of:

1 ACTGAAGCCA GCTCTCTCAC TCTCTTTCTC CACCATGAGG ATCTCTGCCA
 51 CGTTCTGTG CTTGCTGCTC ATAGCCGCTG CTTTCAGCAT CCAAGTGTGG
 101 GCCCAACCAG ATGGGCCCAA TGCATCCACA TGCTGCTATG TCAAGAAACA
 151 AAAGATCCCC AAGAGGAATC TCAAGAGCTA CAGAAGGATC ACCAGTAGTC
 30 201 GGTGTCCCTG GGAAGCTGTT ATCTTCAAGA CAAAGAAGGG CATGGAAGTC
 251 TGTCGTGAAG CCCATCAGAA GTGGGTCGAG GAGGCTATAG CATACTTAGA
 301 CATGAAAACC CCAACTCCAA AGCCTTGAAG AAATGTGCCT GAACAGAAAC
 351 CAACCTAGGA GCCAAGAAGC AAAAATTCCT CACGCTGTT CTTTCTGAGA
 401 ACTGTTGATG AAATGTGTTG ATCACGGTCC TAAGGGATAG GAGCTGTCTG
 35 451 TAGGAATGTG AAACAGTCAC GCCTAAGGAA TGGTCTTTAA GTTATTAATA
 501 TTTTATTTA ATTAGCCATG TACTTTGGTG TGATTTGAAT GTAAAGCTCT

-20-

551 GGAGACCTCA TGTCACCTTA ACATTGTGTT AGCTGCAGAA TTC

which corresponds to the cDNA sequence and derived amino acid sequence human *fic* (growth factor-activated gene). See Heinrich et al., Molecular and Cellular Biology 13: 2020-2030, 1993.

5 In another aspect, the present invention provides a heparanase having the amino acid sequence (SEQ ID NO: 40) of:

Asp Ser Val Ser Ile Phe Ile Thr Cys Cys Phe Asn Val Ile Asn Arg Lys Ile Pro Ile Gln
Arg Leu Glu Ser Tyr Thr Arg Ile Thr Asn Ile Gln Cys Pro Lys Glu Ala Val Ile Phe Lys
Thr Gly Lys Glu Val Cys Ala Asp Pro Lys Glu Arg Trp Val Arg Asp Ser Met Lys His
10 Lys Asp Gln Ile Phe Gln Asn Leu Lys Pro

which corresponds to the cDNA sequence and derived amino acid sequence monocyte chemoattractant protein 2 (MCP-2). See VanDamme et al., J. Exp. Med. 176: 59 - 65, 1992.

The purified heparanase of the present invention, allows for the convenient selection of compounds having anti-heparanase activity (AHA compounds), i.e. inhibitors of heparanase
15 activity (IHA), by measuring inhibition of heparanase activity. Inhibition of heparanase activity can be measured utilizing *in vivo* radiolabeled heparan sulfate/heparin. This ligand is radiolabeled to high specific activity by intraperitoneal injection of 0.5mCi of S-35 sulfate into C57 mice bearing a 1-2 cm basement membrane tumor (EHS; Engelbreth, Holm, Swarm tumor). The tumor is harvested after 16 hours and the heparan sulfate proteoglycan extracted in 4
20 volumes of 6M urea, 20mM Tris pH 6.8, protease inhibitors, 0.15M NaCl and 0.5% triton X-100. The urea extract is chromatographed on an anion exchange column and the proteoglycan is eluted in a linear gradient of NaCl. The radiolabeled proteoglycan is exchanged into a solution of 4.0M guanidine-HCl, 20mM Tris pH 7.4 and applied to a size exclusion column. The proteoglycan peak is pooled and exchanged into 0.15mM NaCl and 20mM Tris pH7.4.

25 Purified, radiolabeled proteoglycan is coupled to commercially available agarose support. A quantitative assay of heparanase activity is constructed with the radiolabeled ligand in a multi-well format. Briefly, known quantities of recombinant heparanase are added to a multi-well plate containing equal amounts of radiolabeled ligand in each well. Enzyme-ligand interaction proceeds overnight and the ligand-agarose complex is recovered by centrifugation. Radioactivity
30 in the liquid phase is determined by scintillation counting and is the measure of enzyme activity. Potential enzyme inhibitors can be evaluated by adding the compound to the solution phase or alternatively adding the assay components to multi-well plates containing preweighed amounts of test compound.

In addition, the purified heparanase of the subject invention can be used for therapeutic
35 wound healing or can be immobilized onto filters and used to degrade heparin from the blood of patients post-surgery.

Wound treatment can be achieved by administration to an afflicted individual an effective amount of a pharmaceutical composition comprising the purified heparanase in combination with a pharmaceutically acceptable, preferably slow releasing, carrier. See. e.g. PCT/US90/04772, incorporated herein by reference.

5 Immobilization onto filters can be achieved by the methods well known in the art including those disclosed by Langer et al. in *Biomaterials: Inter-facial Phenomenon and Applications*, eds. Cooper et al, pp 493-509, 1982 and those described in U.S. Patent No. 4,373,023, 4,863,611 and 5,211,850 (all incorporated herein by reference).

10 The purified heparanase of the subject invention can be prepared by the method described in procedure A or procedure B, but preferably procedure A.

PROCEDURE A

Reverse transcription of the mRNA from activated human leukocyte-derived cells [preferably lymphocytes, neutrophils, platelets, Jurkatt lymphoma cells, Dami cells (Greenberg et al., *Blood* 72:1968-1977, (1988))] is used to prepare the cDNA for the desired heparanase
15 enzyme (preferably SEQ. ID. NO: 1; optionally SEQ. ID. NO: 3, SEQ. ID. NO: 5, SEQ. ID. NO: 7; SEQ. ID. NO: 13, SEQ. ID. NO: 15, SEQ. ID. NO: 17, SEQ. ID. NO: 19, SEQ. ID. NO: 21, SEQ. ID. NO: 23, SEQ. ID. NO: 25, SEQ. ID. NO: 27, SEQ. ID. NO: 29; SEQ. ID. NO: 31, SEQ. ID. NO: 33 or SEQ. ID. NO: 35), employing standard PCR cloning techniques (described in Sambrook et al., in: *Molecular Cloning, A Laboratory Manual*. Second Edition,
20 1989. Cold Spring Harbor Press). The cDNA encoding the heparanase enzyme is cloned into XbaI/BamHI sites in the commercially available baculovirus vector pVL 1392 (Pharmlngen; San Diego, CA). High titer infectious virus is selected for use in infecting sf9 insect cells (Luckow and Summers, *Bio/Technology*. 6,47 1988). Serum-free medium conditioned by infected sf9 cells is collected after 72 hours. This media is the starting material for purification
25 of recombinant heparanase. Serum-free conditioned media is adjusted to contain 20mM Sodium Acetate, pH 5.0, 0.15M NaCl, 1mM reduced glutathione (GSH), 1mM dithiothreitol (DTT) and 10mM beta-octylglucoside. Medium is applied to a column of cation-exchange resin (Pharmacia) and eluted from the column in a linear gradient of NaCl. Fractions containing heparanase are pooled and diluted to a final salt concentration of 0.15M NaCl. To this solution
30 is added 20mM Tris and the pH adjusted to 7.0. The solution is applied to a column of heparin-Sepharose (Pharmacia) and eluted with a linear salt gradient buffered to pH 5.0 with 20mM Sodium Acetate. Heparanase is concentrated to 0.5mg/ml in an Amicon concentrator fitted with a YM-2 membrane and stored at -80 degrees. For optimal activity (greater than 50 units heparanase activity per μ g protein) incubation in the presence of transglutaminase, under
35 reducing conditions, in accordance with the procedure in Example 2, Part C.

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PROCEDURE B

This procedure describes the purification to homogeneity of heparanase (SEQ. ID. NO: 1) from human blood cells or cell lines (such as platelets) under reducing conditions which allow for the occurrence of post-translational modifications that increase the specific activity of heparanase and make it suitable for use in the above described screening assay. The cells are treated with a suitable activator (such as, but not limited to, thrombin or histamine) which allows for the release of enzymes and cytokines from the cell. Reducing agents are added to the supernatant from the activated cells. Suitable reducing agents include dithiothreitol (DTT), dithioerythritol (DTE), reduced glutathione (GSH), and β -mercaptoethanol. The reduced, activated supernatant is chromatographed on a column of immobilized heparin or heparan sulfate under reducing conditions at pH 5, using a salt gradient (such as NaCl, KCl, or other salt) to elute the bound proteins. Fractions containing heparanase activity are pooled and exchanged into any buffer appropriate for the pH of 6.8 and containing 0.15 M NaCl, reducing agents, and non-ionic detergent. This is passed over any suitable anion-exchange column (bed volume of 5 ml or less). The unbound material from this column is adjusted to pH 5 with acid, and is loaded onto any suitable cation-exchange column (bed volume of 5 ml or less), equilibrated in a suitable pH 5 buffer containing 0.15 M NaCl, reducing agents, and non-ionic detergents. The bound protein is eluted from the column with a salt gradient, and the fractions containing heparanase activity are pooled and size fractionated to below 30,000 daltons with 30 K-cut-off membranes. The protein below 30,000 daltons is concentrated by either heparin-sepharose chromatography or by centrifugation through 5 K-cut-off membranes.

The present invention is seen more fully by the examples set forth below.

Example 1: Use of Heparanase as a screen for AHA compounds.

1. Heparan sulfate, metabolically labeled (S-35) to a high-specific activity- as described above for the EHS tumor, prepared by papain digestion of chromatographically purified heparan sulfate proteoglycan is coupled to cyanogen bromide activated Sepharose-6B (Pharmacia) according to manufacturer's instructions.

2. 35 S-Heparan sulfate-Sepharose 6B is resuspended in: 0.15 M NaCl, 0.03% human serum albumin, 10 μ M $MgCl_2$, 10 μ M $CaCl_2$, antiproteolytic agents (1 μ g/ml leupeptin, 2 μ g/ml antipain, 10 μ g/ml benzamidin, 10 units/ml aprotinin, 1 μ g/ml chymostatin, and 1 μ g/ml pepstatin), and 0.05 M Na acetate, pH 5.6 and 5,000 cpm, in a total volume of 200 μ l, are aliquoted into each well of a 96 well plate. To each well is added 5 units of activated heparanase and the digestion allowed to proceed overnight at 37 degrees.

3. Separation of digested product is accomplished by centrifugation of the 96 well plate. The supernatant, containing cleaved heparan sulfate is decanted and quantitated by scintillation counting.

4. Inhibitors of heparanase activity can be introduced into the liquid-phase of the assay.

5. A potential inhibitor of heparanase activity would be identified by its ability to reduce the amount of radiolabeled heparan sulfate released into the supernatant by 50% at a concentration of 1 μ M or less.

Example 2: The preparation of heparanase under reducing conditions as outlined in Procedure B.

Part A:

- Platelet-rich plasma (10^9 platelets/ml; 1800 ml) is obtained from healthy, informed volunteers by plasmapheresis. The plasma is removed from the platelets by centrifugation (Heldin, *et al.*, *Exp. Cell Res.* 109: 429-437, 1977). Platelets suspended in phosphate buffered saline (PBS; 0.1 original volume) are then stimulated with 1 U/ml thrombin for 5 min at 37°C. This concentration of thrombin was reported to release 100% of the heparanase activity from platelets (Oldberg, *et al.*, *Biochemistry* 19: 5755-5762, 1980). Following activation, the thrombin is inactivated by the addition of 100 mM phenylmethylsulfonylfluoride (PMSF), and the platelets are centrifuged at 2000 x g for 30 min at 4°C. The supernatant is stored at -80°C until used for the chromatographic purification of heparanase (Part B).

Part B: Chromatographic purification of heparanase.

1. Heparin-Sepharose Chromatography. Activated platelet supernatants are pooled and adjusted to contain 1 mM GSH and 1 mM DTT. This pool is loaded (2.5 ml/min) onto a column of heparin-sepharose (2.6 x 7.5 cm, 40 ml) equilibrated in 1 mM GSH, 1 mM DTT, 150 mM NaCl, 10 mM NaPO₄, pH 7.4. After loading the sample, the column is washed with 200 ml of 0.15 M NaCl, 1 mM GSH, 1 mM DTT, 10 mM Na acetate, pH 5, followed by 60 ml of 0.35 M NaCl, 1 mM DTT, 1 mM GSH, 10 mM Na acetate, pH 5. The column is then eluted with a 160 ml linear gradient between 0.35 M NaCl and 1.5 M NaCl in the same buffer. Aliquots of each fraction are used for determination of heparanase activity by the "Purification Assay" described later.
2. Anion-exchange chromatography (For example, DEAE-Sephacel, Pharmacia). The 0.9 M - 1.15 M NaCl fractions from the heparin-sepharose column are concentrated using a stirred cell fitted with a PM-10 membrane, and the buffer is exchanged to 0.15 M NaCl, 1 mM DTT, 1 mM GSH, 10 mM β -octylglucoside, 10 mM sodium phosphate, pH 6.8 (8 ml). This sample is loaded onto a 5 ml column of DEAE-Sephacel equilibrated in the same buffer. After loading, the column is washed to baseline absorbance (280 nm) with the equilibration buffer. The flow-through and wash with equilibration buffer are collected as one pool. The column is then eluted with 10 ml of 0.15 M NaCl, 10 mM β -octylglucoside, 1 mM GSH, 1 mM DTT, 10 mM Na acetate, pH 5, followed by 10 ml of 1.5 M NaCl, 10 mM β -octylglucoside, 1 mM GSH, 1 mM

DTT, 10 mM Na acetate, pH 5. Aliquots of each pool are used for determination of heparanase activity by the "Purification Assay".

3. Cation Exchange. The unbound sample from the DEAE-Sephacel column is adjusted to pH 5 with glacial acetic acid and loaded onto a cation exchange column (Poros HS/F, 4.6 mm x 50 mm; PerSeptive Biosystems), pre-equilibrated with 0.15 M NaCl, 1 mM DTT, 1 mM GSH, 10 mM β -octylglucoside, 10 mM Na acetate, pH 5. The flow rate is 3.0 ml/min and 3 ml fractions are collected. After washing the column with 35 ml of equilibration buffer, the column is developed with a 55 ml linear gradient between 0.15 M and 1.5 M NaCl in the same buffer. 10 μ l aliquots of the fractions are used for determination of heparanase activity by the "Purification Assay".

4. Size fractionation to < 30 kD and concentration on immobilized heparin (Hi-trap heparin-sepharose, Pharmacia). The activity from the Poros HS/F column is size fractionated by centrifuging through 30,000 molecular weight cut-off filters (Millipore ultrafree-MC 30,000 NMWL filter units). The < 30 kD pool is diluted to contain 0.15 M NaCl, and is loaded onto a 1 ml Hi-trap heparin column, pre-equilibrated with 0.15 M NaCl, 1 mM DTT, 1 mM GSH, 10 mM Na acetate, pH 5. The column is eluted with 1.2 M NaCl in the same buffer and the single eluted peak contains the heparanase activity.

Part C: Properties of the purified heparanase.

The final yield of heparanase protein from 1850 ml platelet-rich plasma was 2.7 mg.

- 20 Protein concentration was determined by the method of Lowry (*J. Biol. Chem.* 193: 265-275, 1951), or if more precise determinations were required, by amino acid analysis on an amino acid analyzer (Beckman 6300). The overall recovery of activity was 8%, with a 4150-fold purification. The preparation was judged to be homogeneous by the presence of a single band of 9000 daltons on an 18% silver-stained SDS-polyacrylamide gel, run according to the method of Laemmli (*Nature* 227: 680-685, 1970).

The pH optimum of the purified heparanase was determined by conducting the "Purification assay" activity between pH 3.5 and 8.0, using a citrate buffer (pH 3.5 - 6.0), citrate-phosphate buffer (pH 6.5 - 7.0), and phosphate buffer (pH 7.5 - 8.). Heparanase was active between pH 5.0 and 8.0, with the optimum pH at 5.8.

- 30 N-terminal amino acid sequencing of heparanase produced by this procedure was performed using a gas/liquid phase Protein Sequencer (Applied Biosystems Inc. Model 470). Phenylthiodantoin amino acids were resolved and quantitated by an on-line HPLC system (Model 120, Applied Biosystems Inc.) with data analysis on a Nelson Analytical System. N-terminal amino acid sequences of the heparanase produced in this example were 85 %
- 35 SEQ. ID. NO: 9 (namely:

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Asn Leu Ala Lys Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala
 1 5 10 15

Glu Leu Arg),

which is identical to CTAP-III, and 15% SEQ. ID. NO: 10 (namely:

5 Ser Ser Thr Lys Gly Gln Thr Lys Arg Asn Leu Ala Lys Gly Lys Glu),
 1 5 10 15

which is the precursor form, platelet basic protein. Interestingly, the N-terminal sequence of commercial β -thromboglobulin (namely, Calbiochem (Cat. # 605165), Celsus Laboratories (Cat. # 41705), and Haematologic Technologies (Cat. # HBTG-0210), which has low levels of

10 heparanase activity, was 100% SEQ. ID. NO: 11 (namely:

Asn Leu Ala Lys Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala Glu),
 1 5 10 15

indicating that the commercial preparation is actually CTAP-III and not β -thromboglobulin.

Chromatofocusing of the heparanase produced by this procedure results in two peaks of
 15 differing isoelectric points. To perform the chromatofocusing, heparanase is dissolved in 0.025 M imidazole, pH 7.3. The sample is loaded onto a 0.5 x 20 cm column of Polybuffer Exchanger 94 (Pharmacia), equilibrated with 0.025 M imidazole, pH 7.3. Immediately after sample loading, Polybuffer 74 (Pharmacia; 1:8, pH 4) is pumped onto the column at 0.5 ml/min. 2 ml fractions are collected, and the pH of each fraction is determined by a narrow range (pH 4-
 20 7) pH paper. Aliquots of each fraction are used to determine heparanase activity by the "Purification Assay." All of the activity is associated with an absorbance (280 nm) peak that eluted at pH 4.8 to 5.1, representing approximately 10% of total protein, while 90% of the protein is eluted at pH 7.3 and is inactive. Aliquots of each protein peak are separated from the ampholytes by C_4 reverse phase chromatography. The peak that eluted from the chromato-
 25 focusing column at pH 7.3 has N-terminal sequences for platelet basic protein and the processed form, CTAP-III. The peak that is eluted from the chromatofocusing column at pH 4.8 - 5.1 also contains the sequences of platelet basic protein and the processed form, CTAP-III. All of the platelet basic protein processed forms have pI's that are calculated and reported to be greater than 7.6. Thus, the heparanase activity resides in the platelet basic protein and/or the processed
 30 form, CTAP-III that is modified such that the pI is lowered to 4.8 - 5.1.

Heparanase obtained after chromatofocusing exhibited a specific activity of 80 units/ μ g protein, using the "Purification Assay." This represents a 1000-fold increase in the specific activity compared to the commercial protein (β -thromboglobulin; 0.075 units/ μ g protein).

The modification that may be responsible for the lower isoelectric point of active
 35 heparanase is ADP-ribosylation. ADP-ribosylation (Adenine diphosphate-ribosylation) is a post-translational modification of proteins or DNA in which the ADP-ribose group of NAD (Nicotinamide adenine dinucleotide) is enzymatically transferred to proteins or DNA. Since this

modification adds two negatively charged phosphate groups to a molecule, it would result in a lower isoelectric point. Activated platelet supernatants were incubated in 1 mM DTT, 2 mM MgCl_2 , 100 mM HEPES, pH 7.4, and 0.5 μM [^{32}P]NAD (Specific activity = 1000 Ci/mmol). The labeled proteins were separated by SDS-polyacrylamide gel electrophoresis on an 18% gel, transferred to PVDF (polyvinylidene difluoride) membrane, and exposed to X-ray film. The autoradiogram demonstrates the incorporation of [^{32}P] into a protein of 8000 daltons. The PVDF membrane was immunoblotted with the anti-Peptide C antisera (1:1500 in PBS containing 5% dry milk, 0.05% Tween-20, 0.15 M NaCl, 20 mM Tris, pH 7.4, 2 hours at room temperature, followed by incubation with peroxidase-labeled goat anti-chicken IgG (1:500 in above buffer, 1 hour room temperature), and reacted with a peroxidase substrate. The immunoblot revealed that the 8000 dalton that was labeled with [^{32}P] was CTAP-III/heparanase. The addition of 200 μM sodium nitroprusside, a spontaneous releaser of nitric oxide, to the ADP-ribosylation reaction resulted in 5-fold more incorporation of [^{32}P] label into CTAP-III/heparanase, suggesting that this modification can be regulated in vivo by nitric oxide.

Finally, in an analogous manner to that of glyceraldehyde-3-phosphate dehydrogenase, another platelet ADP-ribosylated glycolytic enzyme (Zhang and Snyder, *Proc. Natl. Acad. Sci. USA* 89: 9382-9385), it was determined that CTAP-III/heparanase has an auto-ADP-ribosylation activity, since the [^{32}P]-ADP-ribosylation of CTAP-III/heparanase occurs in reactions where the only protein present is commercial CTAP-III or purified heparanase. Other chemokine family members tested, which includes IP-1-, IL-8, gro- α , and MCAF, also have auto-ADP-ribosylation activity.

It is contemplated that the high specific activity of CTAP-III/heparanase is a consequence of ADP-ribosylation of the enzyme in the presence of nitric oxide. It is further contemplated that the action of transglutaminase on the ADP-ribosylated enzyme will lead to further increase in the specific activity.

An amino acid composition of the heparanase produced in Example 2 gave the expected amino acid composition for CTAP-III and N-terminal sequencing revealed sequences for platelet basic protein and the processed form, CTAP-III, confirming that the heparanase activity is contained in this set of processed proteins and is not due to a minor contaminant. The presence of heparanase activity in three commercial sources of β -thromboglobulin also confirms this conclusion. In addition, polyclonal antibodies to β -thromboglobulin were found to precipitate 30 - 70% of the heparanase activity in three separate experiments, providing additional confirmation.

The activation of heparanase with transglutaminase (prepared in accordance with Example 2, Part B) results in a substantial (about 13-fold) increase in the specific activity of the enzyme. The heparanase (2 μl at 56 nM) obtained by Example 2, Part B is treated with either

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transglutaminase from guinea pig liver (4 mU; Sigma) or with Factor XIII (1 µg; Celsus Laboratories, Inc.), the blood coagulation factor that is activated by treatment with 5 units of thrombin at 37 degrees for 30 minutes. Heparanase is activated by incubation of either 2mU liver transglutaminase or 5 units of activated Factor XIII in the presence of 0.1M NaAcetate buffer at pH 6.0 containing 1mM reduced glutathione and 1mM CaCl for 35 minutes at 37 degrees. Treatment of heparanase with either type of transglutaminase results in a substantial increase in the specific activity of the heparanase.

The high degree of sequence identity between CTAP-III and Interleukin-8, a CXC chemokine family member, assures that an essentially identical folding pattern will be shared by the two proteins. Since the 3-dimensional structure of Interleukin-8 is known (Clowes, *et al.*, *Biochemistry* 29: 1689-1696, 1990; Baldwin, *et al.*, *J. Biol. Chem.* 265: 6851-6853), one can model the same for CTAP-III. Such a model can serve to direct research into rationally designed IHA and to help explain the action of transglutaminase in activating the CTAP-III.

Part D: Purification Assay for Heparanase Activity

Heparanase activity from platelets or column fractions is detected by its ability to digest the ≥ 70 kD ^{35}S -HSPG to produce lower molecular weight products. Each digest contains 10 µl sample, ^{35}S -HSPG (2000 cpm), 0.15 M NaCl, 0.03% human serum albumin, 10 µM MgCl_2 , 10 µM CaCl_2 , antiproteolytic agents (1 µg/ml leupeptin, 2 µg/ml antipain, 10 µg/ml benzamidin, 10 units/ml aprotinin, 1 µg/ml chymostatin, and 1 µg/ml pepstatin), and 0.05 M Na acetate, pH 5.6 in a total volume of 300 µl. Digests are carried out for 3 to 21 h. The presence of lower molecular weight radiolabeled products is detected by centrifugation through 30,000 MW-cutoff filters. The digests containing 2000 cpm of ^{35}S -HSPG (> 70 K) are centrifuged through 30,000 molecular weight cut-off filters (Millipore Ultrafree-MC 30,000 NMWL filter units). ^{35}S -HSPG degradation is evident by the presence of radioactivity in the filtrate that passed through the 30 K membrane; this heparanase activity is expressed as the % of total cpm < 30 K for a given digest. Analysis of heparan sulfate degradation by this method is quick and reproducible. 1 unit of heparanase activity is defined as 1% cpm < 30 K per h. For pH optimum determination, the 0.1 M Na acetate buffer is replaced by 50 mM citrate, citrate-phosphate, or phosphate buffer at varying pH's. For samples from chromatographic steps performed under reducing conditions (1 mM GSH, 1 mM DTT), the concentration of a thiol oxidant (diamide) needed for optimum activity is determined. This concentration (100 µM diamide) is added to all assay tubes when reduced samples are assayed.

Preparation of ^{35}S -HSPG (>70 K) for use in the "Purification Assay."

^{35}S -HSPG (>70 K) is prepared from mice bearing a basement membrane tumor that overproduces HSPG (EHS tumor), using modifications of the method of Ledbetter, *et al.*, 1987. Briefly, the radiolabeled HSPG was prepared by injecting C57BL mice bearing the EHS tumor

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- (Orkin, et.al., 1977) with sodium [^{35}S]sulfate (0.5 mCi/mouse) 18 h before harvesting the tumor. The HSPG is extracted from the weighed tumor with 6 volumes (w/v) of Buffer A (3.4 M NaCl, 0.1 M 6-aminohexanoic acid, 0.04 M EDTA, 0.008 M *N*-ethylmaleimide, 0.002 M PMSF, and 0.05 M Tris-HCl, pH 6.8), by homogenization with a Polytron for 30 s, followed by stirring at 4°C for 1 h. Insoluble material is collected by centrifugation (12,000 x g for 10 min), and the supernatant is discarded. The insoluble residue is reextracted with 2 volumes (original tumor weight) of Buffer A for 30 min with stirring at 4°C. Insoluble material is again collected by centrifugation, and the supernatant fraction is discarded. The insoluble material is then suspended in 6 volumes of Buffer B (6 M urea, 0.1 M 6-aminohexanoic acid, 0.04 M ethylenediaminetetraacetic acid (EDTA), 0.002 M PMSF, and 0.05 M Tris-HCl, pH 6.8), homogenized with an electric homogenizer (Polytron) for 30 s, and stirred for 2 h at 4°C. The mixture is centrifuged to remove insoluble material, and the supernatant is retained. The insoluble material is reextracted with 2 volumes of Buffer B. The mixture is centrifuged, and the supernatant is combined with the previous supernatant.
- ^{35}S -HSPG is isolated from the Buffer B supernatant by sequential chromatography on anion exchange and gel filtration columns. The Buffer B supernatant is dialyzed overnight against 10 volumes of 6 M urea, 0.15 M NaCl, 0.05 M Tris-HCl, pH 6.8, and is adjusted to contain 0.5% non-ionic detergent (Triton X-100). This supernatant (from 11 g tumor) is chromatographed on a 30 ml column of anion exchange resin (DEAE-Sephacel) equilibrated with 6 M urea, 0.15 M NaCl, 0.05% Triton X-100, 0.05 M Tris-HCl, pH 6.8. After loading the supernatant and washing with the equilibration buffer, the column is developed with a 250 ml linear gradient between 0.15 M NaCl and 1.15 M NaCl (Flow = 2.0 ml/min). Fractions are sampled for radioactivity, and those containing the $^{35}\text{SO}_4$ label that elutes from the DEAE-Sephacel between 0.4 M and 0.8 M NaCl are pooled. The proteoglycan is precipitated by the addition of 4 volumes of 100% EtOH at -20°C overnight. The precipitate is collected by centrifugation and is solubilized in 1 ml of Buffer C (4 M Gu-HCl, 20 mM Tris-HCl, pH 7.2). This solubilized pellet is used for chromatography on a calibrated gel filtration column (1.0 x 50 cm column of Superose 6; Pharmacia) equilibrated in Buffer C (Flow = 0.5 ml/min). Fractions are sampled for radioactivity, and those containing the $^{35}\text{SO}_4$ label that elutes with a molecular weight ≥ 70 kD were pooled. The proteoglycan is precipitated with 100% EtOH as described above. The pellet is dissolved in 3 ml PBS, and dialyzed against 3 x 100 volumes of PBS. Each preparation of ^{35}S -HSPG is confirmed to be $\geq 98\%$ heparan sulfate by susceptibility to low pH nitrous acid degradation (Shiveley and Conrad, *Biochemistry* 15: 3932-3942, 1976).
- Example 3: Preparation of cDNA encoding Heparanase.
- Media is removed from cultured HEL (HEL 92.1.7; Human erythroleukemia; ATCC No. TIB 180) cells stimulated with 10nM phorbol 12-myristate 13-acetate (Sigma Chemical Co., St.

Louis, MO) and the cells scraped from the dish and pelleted by centrifugation. The pellet is extracted with 200µl of TRI reagent (Molecular Research Center Inc. Cincinnati, OH) and the total cellular RNA is prepared according to the manufacturer's instructions. To prepare first strand synthesis the reverse transcriptase reaction was performed with 10µl of total cellular RNA in the presence of 4µl of 5x transcriptase buffer (Bethesda Research Laboratories, Gaithersburg, MD), 1µl 0.2mM DTT, 4µl random hexanucleotides (Amersham Corp. Arlington Heights, ILL), and 1µl 10mM dNTP (BRL). This solution is heated to 95 degrees C for 5 minutes and then placed on ice. To this is added 1µl RNasin and 1µl reverse transcriptase (M-MLV), (Promega, Madison WI). This is incubated at 37 degrees for 60 minutes and then placed on ice. The polymerase chain reaction is carried out as follows. To 3µl of the first strand (above) is added 1µl of each Primer (see below), 77µl of water 10µl 10x PCR buffer (Perkin Elmer Cetus, Norwalk CT) and 2µl each dNTP. This solution is heat denatured at 95 degrees C and 1µl Amplitaq DNA polymerase (Perkin Elmer Cetus) is added. Hybridization temperature begins at 72 degrees and is lowered by one degree per cycle until reaching 55 degrees. Each hybridization step is followed with a constant elongation temperature of 72 degrees. Upon completion the solution is left at 0 degrees until storage at -20 degrees. The products of the PCR reaction are electrophoresed on 3% NuSieve, 1% agarose gels and bands of expected size are excised and purified by standard procedures.

Primers:

20 Platelet Basic Protein: TGG ACT AGT ATG TCC TCC ACC AAA GGA CAA ACT AA
 CTAP III: TGG ACT AGT ATG AAC TTG GCG AAA GAG GA
 B-thromboglobulin: TGG ACT AGT ATG GGC AAA GAG GAA AGT CTA GAC AG
 NAP-2: TGG ACT AGT ATG GAA CTC CGC TGC ATG TGT ATA AA

Example 4: Preparation of cDNA encoding Heparanase.

25 Media is removed from cultured leukocyte-derived cells [e.g., lymphocytes, neutrophils, platelets, Jurkatt lymphoma cells, Dami cells (Greenberg et al., Blood 72:1968-1977, (1988)], stimulated with Concanavalin A or phorbol 12-myristate 13 acetate (Sigma Chemical Co., St. Louis, MO) and the cells scraped from the dish and pelleted by centrifugation. The pellet is extracted with 200µl of TRI reagent (Molecular Research Center Inc. Cincinnati, OH) and the total cellular RNA is prepared according to the manufacturer's instructions. To prepare first strand synthesis the reverse transcriptase reaction was performed with 10µl of total cellular RNA in the presence of 4µl of 5x transcriptase buffer (Bethesda Research Laboratories, Gaithersburg, MD), 1µl 0.2mM DTT, 4µl random hexanucleotides (Amersham Corp. Arlington Heights, ILL), and 1µl 10mM dNTP (BRL). This solution is heated to 95 degrees C for 5 minutes and then placed on ice. To this is added 1µl RNasin and 1µl reverse transcriptase (M-MLV), (Promega,

-30-

- Madison WI). This is incubated at 37 degrees for 60 minutes and then placed on ice. The polymerase chain reaction is carried out as follows. To 3 μ l of the first strand (above) is added 1 μ l of each Primer (see below), 77 μ l of water 10 μ l 10x PCR buffer (Perkin Elmer Cetus, Norwalk CT) and 2 μ l each dNTP. This solution is heat denatured at 95 degrees C and 1 μ l
- 5 Amplitaq DNA polymerase (Perkin Elmer Cetus) is added. Hybridization temperature begins at 72 degrees and is lowered by one degree per cycle until reaching 55 degrees. Each hybridization step is followed with a constant elongation temperature of 72 degrees. Upon completion the solution is left at 0 degrees until storage at -20 degrees. The products of the PCR reaction are electrophoresed on 3% NuSieve, 1% agarose gels and bands of expected size
- 10 are excised and purified by standard procedures.

Primers:

- Platelet Basic Protein: TGG ACT AGT ATG TCC TCC ACC AAA GGA CAA ACT AA
 CTAP III: TGG ACT AGT ATG AAC TTG GCG AAA GAG GA
 B-thromboglobulin: TGG ACT AGT ATG GGC AAA GAG GAA AGT CTA GAC AG
 15 NAP-2: TGG ACT AGT ATG GAA CTC CGC TGC ATG TGT ATA AA

All temperatures expressed throughout the subject specification are in degrees Centigrade.

- The cDNA encoding heparanase is preferably cloned into a vector designed for expression in eukaryotic cells, rather than into a vector designed for expression in prokaryotic cells (e.g. *E. coli*). Eukaryotic cells are preferred for expression of genes obtained from higher
- 20 eukaryotes because the signals for synthesis, processing, and secretion of these proteins are usually recognized, whereas this is often not true for prokaryotic hosts (Ausubel, *et al.*, ed., in Short Protocols in Molecular Biology, 2nd edition, John Wiley & Sons, publishers, pg.16-49, 1992.). Eukaryotic hosts may include, but are not limited to, the following: insect cells, African green monkey kidney cells (COS cells), Chinese hamster ovary cells (CHO cells), and
- 25 Murine 3T3 fibroblasts.

Experiments demonstrating that a synthetic peptide of CTAP-III/NAP-2 or antisera raised against a synthetic peptide of CTAP-III/NAP-2 inhibit the heparanase activity of CTAP-III/NAP-2 suggest that the amino acids participating in enzymatic catalysis are contained in a C-terminal region of the enzyme.

- 30 **Peptide Synthesis:** A C-terminal peptide contained within the sequences known for CTAP-III (SEQ ID NO: 1), Platelet Basic Protein (SEQ ID NO: 3), β -thromboglobulin (SEQ ID NO: 5), and NAP-2 (SEQ ID NO: 7), and an N-terminal peptide contained within the sequences known for CTAP-III (SEQ ID NO: 1) and Platelet Basic Protein (SEQ ID NO: 3) were synthesized according to standard procedures. The N-terminal peptide has the following sequence (SEQ ID
- 35 NO: 41: Asn Leu Ala Lys Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Cys, in which the final Cys residue was added to regions of known sequence (SEQ ID NOS: 1,3) for the purpose of

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conjugation to a carrier protein. The C-terminal peptide has the following sequence (SEQ ID NO: 42): Cys Asn Gln Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys encoded by the cDNA sequence (SEQ ID NO: 43) of TGCAACCAAG TCGAAGTGAT AGCCACACTG AAGGATGGGA

- 5 GGAAATCTG CCTGGACCCA GATGCTCCCA GAATCAAGAA AATTGTACAG
AAAAAA. These peptides (SEQ ID NOS: 41 and 42) were produced by stepwise solid phase peptide synthesis on an Applied Biosystems 430A Peptide Synthesizer. The 9-fluorenylmethyloxycarbonyl (Fmoc) group was used as the N^α amino protecting group, and temporary side-chain protecting groups were as follows: Arg (Pmc), Asn (Trt), Asp (OtBu), Gln (Trt), Glu (OtBu), His (Trt), Lys (Boc), Ser (tBu), Thr (tBu). Each residue was single coupled using a HBTU/NMP protocol and capped with acetic anhydride before the next synthesis cycle. After removal of the N-terminal Fmoc group, temporary side-chain protecting groups were removed and the peptide cleaved from the resin by treatment with 95% TFA/5% scavengers (ethyl methyl sulfide/anisole/1,2-ethanedithiol, 1:3:1) for two hours at room temperature. The
- 10 crude peptides were precipitated from the cleavage solution with cold diethyl ether. The precipitated peptide was collected on a sintered glass funnel, washed with diethyl ether, dissolved in dilute acetic acid, evaporated to dryness under reduced pressure, and the residue was redissolved and lyophilized from glacial acetic acid. The crude peptides were purified by preparative reverse phase chromatography on a Phenomenex C-18 column (22.5 x 250 mm)
- 15 using a water/acetonitrile gradient, each phase containing 0.1% trifluoroacetic acid (TFA). Clean fractions, as determined by analytical HPLC, were pooled, the acetonitrile was evaporated under reduced pressure, and the aqueous solution was lyophilized. The purified peptides were characterized by time of flight or FAB mass spectroscopy.

- Further, SEQ ID NO: 42 can be produced by recombinant DNA methodology as stated
- 25 in Procedure A (page 21).

- Antisera Production:** The synthetic peptides of CTAP-III/NAP-2 were conjugated to keyhole limpet hemocyanin utilizing a maleimide-activated carrier protein (Pierce Chemical Co. #77107). 300 µg of conjugated peptides were injected into chickens using Freund's complete adjuvant. The antisera were collected 5 weeks after initial immunization. Specific recognition by the
- 30 antisera of commercial CTAP-III (2.5 µg, (Celsus Laboratories Inc., Cincinnati, Ohio; Cat #. 41705), isolated heparanase (1.5 µg), and 10 µl of the platelet supernatant used for purification was achieved by separating the proteins on a reducing 18% polyacrylamide gel (Novex), transferring to nitrocellulose, and incubating with the pre-immune or antisera (1:1500), followed by incubation with a peroxidase labeled goat anti-chicken IgG (1:500; Kierkegaard and Perry) in
- 35 the presence of PBS containing 5% dry milk and 0.05% Tween-20. The pre-immune sera did not recognize 7 - 10 kD proteins in the commercial CTAP-III, isolated heparanase, or platelet

supernatants.

Inhibition of heparanase activity by the C-terminal synthetic peptide (SEQ ID NO: 42) or

antisera: For experiments designed to determine whether the peptide antisera was able to inhibit heparanase activity, the pre-immune and antisera were exchanged into 0.15M NaCl,

- 5 0.01M sodium phosphate buffer, pH 7.4 (PBS) using a 100 kD cut-off membrane in order to remove low molecular weight chicken heparanase normally present in the serum. Aliquots of isolated heparanase (15 ng) were pre-incubated for 30 min with 2 μ l of either pre-immune or anti-CTAP-III antisera before adding the 35 S-HSPG to determine heparanase activity. In the presence of the pre-immune sera, the isolated protein had 14.3 ± 0.1 units of heparanase activity, while in the presence of the C-terminal peptide antisera, only 0.8 ± 0.2 units of heparanase were detected ($p < 0.001$; results confirmed in a second experiment). The N-terminal peptide antiserum was not able to neutralize the heparanase activity. Similar results were obtained when the ability of the synthetic peptides to neutralize heparanase activity was examined. Heparanase assays conducted with 3 nM enzyme, 47 nM 35 S-HSPG substrate, and varying concentrations of peptides showed that heparanase activity was only 5% of control values in the presence of 250 μ M C-terminal peptide. By contrast, heparanase activity in the presence of 250 μ M of either the N-terminal peptide or an unrelated peptide (PLALWAR) was 67% of control values. The ability of both the C-terminal peptide (SEQ ID NO: 42) or antisera from a chicken immunized with the C-terminal synthetic peptide to neutralize heparanase activity demonstrates conclusively that
- 10 20 CTAP-III and NAP-2 possess heparanase activity, and suggests that the C-terminal region is essential for catalysis. Modeling of this domain (SEQ ID NO: 42) can be used in the identification of potent peptide-mimetic compounds capable of inhibiting this enzyme activity.

- Computer assisted modeling can be accomplished using programs for automated docking of molecules within 3D databases, as described in DesJarlais, R.L., Sheridan, R.P., Seibel, G.L., Dixon, J.S., Kuntz, I.D., Venkataraghavan, R., "Using shape complementarity as an initial screen in designing ligands for a receptor binding site of known three-dimensional structure"; J. Med. Chem. 31:722-729, 1988. Also, automated de novo construction of ligands that can bind the catalytic site as described in Moon, J.B., Howe, W.J., "Computer design of bioactive molecules: a method for receptor-based de novo ligand design"; Proteins: Struct., Funct., and Genetics,
- 25 30 11:314-328, 1981.

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SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

- (i) APPLICANT: Hoogewerf, Arlene J.
Ledbetter, Steven R.
- 10 (ii) TITLE OF INVENTION: USE OF HEPARANASE TO IDENTIFY AND
ISOLATE ANTI-HEPARANASE COMPOUNDS
- (iii) NUMBER OF SEQUENCES: 43
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: The Upjohn Company, Intellectual Property Law
(B) STREET: 301 Henrietta
(C) CITY: Kalamazoo
(D) STATE: MI
20 (E) COUNTRY: USA
(F) ZIP: 49001
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
25 (B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
30 (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
35 (A) NAME: Jameson, William G.
(B) REGISTRATION NUMBER: 27,199
(C) REFERENCE/DOCKET NUMBER: 4731.1 CP
- (ix) TELECOMMUNICATION INFORMATION:
40 (A) TELEPHONE: 616/385-7561
(B) TELEFAX: 616/385-6897
(C) TELEX: 224401

45 (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
50 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Leu | Ala | Lys | Gly | Lys | Glu | Glu | Ser | Leu | Asp | Ser | Asp | Leu | Tyr | Ala |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Glu | Leu | Arg | Cys | Met | Cys | Ile | Lys | Thr | Thr | Ser | Gly | Ile | His | Pro | Lys |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Asn | Ile | Gln | Ser | Leu | Glu | Val | Ile | Gly | Lys | Gly | Thr | His | Cys | Asn | Gln |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Val | Glu | Val | Ile | Ala | Thr | Leu | Lys | Asp | Gly | Arg | Lys | Ile | Cys | Leu | Asp |
| | | 50 | | | | 55 | | | | | | 60 | | | |

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Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly
 65 70 75 80
 Asp Glu Ser Ala Asp
 85

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 255 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AACTTGGCGA AAGGCAAAGA GGAAAGTCTA GACAGTGACT TGTATGCTGA ACTCCGCTGC 60
 ATGTGTATAA AGACAACCTC TGGAATTCAT CCCAAAACA TCCAAAGTTT GGAAGTGATC 120
 GGGAAAGGAA CCCATTGCAA CCAAGTCGAA GTGATAGCCA CACTGAAGGA TGGGAGGAAA 180
 ATCTGCCTGG ACCCAGATGC TCCAGAATC AAGAAAATTG TACAGAAAAA ATTGGCAGGT 240
 GATGAATCTG CTGAT 255

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Ser Thr Lys Gly Gln Thr Lys Arg Asn Leu Ala Lys Gly Lys Glu
 1 5 10 15
 Glu Ser Leu Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile
 20 25 30
 Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser Leu Glu Val
 35 40 45
 Ile Gly Lys Gly Thr His Cys Asn Gln Val Glu Val Ile Ala Thr Leu
 50 55 60
 Lys Asp Gly Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys
 65 70 75 80
 Lys Ile Val Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala Asp
 85 90

(2) INFORMATION FOR SEQ ID NO:4:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 282 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

-35-

TCCTCCACCA AAGGACAAAC TAAGAGAAAC TTGGCGAAAG GCAAAGAGGA AAGTCTAGAC 60
 AGTGACTTGT ATGCTGAACT CCGCTGCATG TGTATAAAGA CAACCTCTGG AATTCATCCC 120
 5 AAAAACATCC AAAGTTTGA AGTGATCGGG AAAGGAACCC ATTGCAACCA AGTCGAAGTG 180
 ATAGCCACAC TGAAGGATGG GAGGAAAATC TGCCTGGACC CAGATGCTCC CAGAATCAAG 240
 AAAATTGTAC AGAAAAAATT GGCAGGTGAT GAATCTGCTG AT 282

10 (2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:
 15 (A) LENGTH: 81 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys
 1 5 10 15
 25 Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser
 20 25 30
 30 Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn Gln Val Glu Val Ile
 35 40 45
 Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro
 50 55 60
 35 Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala
 65 70 75 80
 Asp

40 (2) INFORMATION FOR SEQ ID NO:6:

(1) SEQUENCE CHARACTERISTICS:
 45 (A) LENGTH: 243 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGCAAAGAGG AAAGTCTAGA CAGTGACTTG TATGCTGAAC TCCGCTGCAT GTGTATAAAG 60
 55 ACAACCTCTG GAATTCATCC CAAAACATC CAAAGTTTGG AAGTGATCGG GAAAGGAACC 120
 CATTGCAACC AAGTCGAAGT GATAGCCACA CTGAAGGATG GGAGGAAAT CTGCCTGGAC 180
 60 CCAGATGCTC CCAGAATCAA GAAATTGTA CAGAAAAAAT TGGCAGGTGA TGAATCTGCT 240
 GAT 243

(2) INFORMATION FOR SEQ ID NO:7:

65 (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 69 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

5 Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys
 1 5 10 15
 Asn Ile Gln Ser Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn Gln
 20 25 30
 10 Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp
 35 40 45
 Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly
 50 55 60
 15 Asp Glu Ser Ala Asp
 65

(2) INFORMATION FOR SEQ ID NO:8:

20 (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 207 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 25 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

30 GAAGTCCGCT GCATGTGTAT AAAGACAACC TCTGGAATTC ATCCCAAAAA CATCCAAAGT 60
 TTGGAAGTGA TCGGGAAAGG AACCCATTGC AACCAAGTCG AAGTGATAGC CACACTGAAG 120
 35 GATGGGAGGA AAATCTGCCT GGACCCAGAT GCTCCAGAA TCAAGAAAAT TGTACAGAAA 180
 AAATTGGCAG GTGATGAATC TGCTGAT 207

(2) INFORMATION FOR SEQ ID NO:9:

40 (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

50 Asn Leu Ala Lys Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala
 1 5 10 15
 Glu Leu Arg

(2) INFORMATION FOR SEQ ID NO:10:

55 (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

65 Ser Ser Thr Lys Gly Gln Thr Lys Arg Asn Leu Ala Lys Gly Lys Glu
 1 5 10 15

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(2) INFORMATION FOR SEQ ID NO:11:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Asn Leu Ala Lys Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala
 1 5 10 15
 Glu

(2) INFORMATION FOR SEQ ID NO:12:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 101 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ser Ser Ala Ala Gly Phe Cys Ala Ser Arg Pro Gly Leu Leu Phe
 1 5 10 15
 Leu Gly Leu Leu Leu Leu Pro Leu Val Val Ala Phe Ala Ser Ala Glu
 20 25 30
 Ala Glu Glu Asp Gly Asp Leu Gln Cys Leu Cys Val Lys Thr Thr Ser
 35 40 45
 Gln Val Arg Pro Arg His Ile Thr Ser Leu Glu Val Ile Lys Ala Gly
 50 55 60
 Pro His Cys Pro Thr Ala Gln Leu Ile Ala Thr Leu Lys Asn Gly Arg
 65 70 75 80
 Lys Ile Cys Leu Asp Leu Gln Ala Pro Leu Tyr Lys Lys Ile Ile Lys
 85 90 95
 Lys Leu Leu Glu Ser
 100

(2) INFORMATION FOR SEQ ID NO:13:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 439 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCGCAGCATG AGCTCCGCAG CCGGGTTCTG CGCCTCACGC CCCGGGCTGC TGTTCCTGGG 60
 GTTGCTGCTC CTGCCACTTG TGGTCGCCTT CGCCAGCGCT GAAGCTGAAG AAGATGGGGA 120
 CCTGCAGTGC CTGTGTGTGA AGACCACCTC CCAGGTCCGT CCCAGGCACA TCACCAGCCT 180

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GGAGGTGATC AAGGCCGGAC CCCACTGCCC CACTGCCCAA CTGATAGCCA CGCTGAAGAA 240
 TGGAAGGAAA ATTTGCTTGG ACCTGCAAGC CCCGCTGTAC AAGAAAATAA TTAAGAAACT 300
 5 TTTGGAGAGT TAGCTACTAG CTGCCTACGT GTGTGCATTT GCTATATAGC ATACTTCTTT 360
 TTTCCAGTTT CAATCTAACT GTGAAAGAAA CTTCTGATAT TTGTGTTATC CTTATGATTT 420
 TAAATAAACA AAATAAATC 439

10 (2) INFORMATION FOR SEQ ID NO:14:

(1) SEQUENCE CHARACTERISTICS:
 15 (A) LENGTH: 101 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

25 Met Asn Gln Thr Ala Ile Leu Ile Cys Cys Leu Ile Phe Leu Thr Leu
 1 5 10 15
 Ser Gly Ile Gln Gly Val Pro Leu Ser Arg Thr Val Arg Cys Thr Cys
 20 25 30
 30 Ile Ser Ile Ser Asn Gln Pro Val Asn Pro Val Asn Pro Arg Ser Leu
 35 40 45
 Glu Lys Leu Glu Ile Ile Pro Ala Ser Gln Phe Cys Pro Arg Val Glu
 50 55 60
 35 Ile Ile Ala Thr Met Lys Lys Lys Gly Glu Lys Arg Cys Leu Asn Pro
 65 70 75 80
 40 Glu Ser Lys Ala Ile Lys Asn Leu Leu Lys Ala Val Ser Lys Glu Met
 85 90 95
 Ser Lys Arg Ser Pro
 100

45 (2) INFORMATION FOR SEQ ID NO:15:

(1) SEQUENCE CHARACTERISTICS:
 50 (A) LENGTH: 650 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAGACATTCC TCAATTGCTT AGACATATTC TGAGCCTACA GCAGAGGAAC CTCCAGTCTC 60
 60 AGCACCATGA ATCAAACCTGC GATTCTGATT TGCTGCCTTA TCTTCTGAC TCTAAGTGGC 120
 ATTCAAGGAG TACCTCTCTC TAGAACCCTA CGCTGTACCT GCATCAGCAT TAGTAATCAA 180
 CCTGTTAATC CAAGGTCTTT AGAAAAACTT GAAATTATTC CTGCAAGCCA ATTTTGTCCA 240
 65 CGTGTGAGA TCATTGCTAC AATGAAAAAG AAGGGTGAGA AGAGATGTCT GAATCCAGAA 300
 TCGAAGGCCA TCAAGAATTT ACTGAAAGCA GTTAGCAAGG AAATGTCTAA AAGATCTCCT 360

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TAAAACCAGA GGGGAGCAAA ATCGATGCAG TGCTTCCAAG GATGGACCAC ACAGAGGCTG 420
 CCTCTCCCAT CACTTCCCTA CATGGAGTAT ATGTCAAGCC ATAATTGTTC TTAGTTTGCA 480
 5 GTTACACTAA AAGGTGACCA ATGATGGTCA CCAAATCAGC TGCTACTACT CCTGTAGGAA 540
 GGTTAATGTT CATCATCCTA AGCTATTGAG TAATAACTCT ACCCTGGCAC TATAATGTAA 600
 GCTCTACTGA GGTGCTATGT TCTTAGTGGA TGTCTGACC CTGCTTCAAA 650

10 (2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 amino acids
 15 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

25 Met Ala Arg Ala Ala Leu Ser Ala Ala Pro Ser Asn Pro Arg Leu Leu
 1 5 10 15
 Arg Val Ala Leu Leu Leu Leu Leu Val Ala Ala Gly Arg Arg Ala
 20 25 30
 30 Ala Gly Ala Ser Val Ala Thr Glu Leu Arg Cys Gln Cys Leu Gln Thr
 35 35 40 45
 Leu Gln Gly Ile His Pro Lys Asn Ile Gln Ser Val Asn Val Lys Ser
 50 55 60
 Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys Asn
 65 70 75 80
 40 Gly Arg Lys Ala Cys Leu Asn Pro Ala Ser Pro Ile Val Lys Lys Ile
 85 90 95
 Ile Glu Lys Met Leu Asn Ser Asp Lys Ser Asn
 100 105

45 (2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1050 base pairs
 50 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTCGCCAGCT CTTCCGCTCC TCTACAGCC GCCAGACCCG CCTGCTGAGC CCCATGGCCC 60
 60 GCGCTGCTCT CTCCGCCGCC CCCAGCAATC CCCGGCTCCT GCGAGTGGCA CTGCTGCTCC 120
 TGCTCCTGGT AGCCGCTGGC CGCGCGCAG CAGGAGCGTC CGTGGCCACT GAACTGCGCT 180
 GCCAGTGCTT GCAGACCTG CAGGGAATTC ACCCAAGAA CATCCAAAGT GTGAACGTGA 240
 65 AGTCCCCCGG ACCCACTGC GCCCAAACCG AAGTCATAGC CAACTCAAG AATGGGCGGA 300
 AAGCTTGCCT CAATCCTGCA TCCCCATAG TTAAGAAAAT CATCGAAAAG ATGCTGAACA 360

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GTGACAAATC CAACTGACCA GAAGGGAGGA GGAAGCTCAC TGGTGGCTGT TCCTGAAGGA 420
 GGCCCTGCCC TTATAGGAAC AGAAGAGGAA AGAGAGACAC AGCTGCAGAG GCCACCTGGA 480
 5 TTGTGCCTAA TGTGTTTGAG CATCGCTTAG GAGAAGTCTT CTATTTATTT ATTTATTCAT 540
 TAGTTTTGAA GATTCTATGT TAATATTTTA GGTGTAAAAT AATTAAGGGT ATGATTAAC 600
 CTACCTGCAC ACTGTCCTAT TATATTCATT CTTTTTGAAA TGTCAACCCC AAGTTAGTTC 660
 10 AATCTGGATT CATATTTAAT TTGAAGGTAG AATGTTTTCA AATGTTCTCC AGTCATTATG 720
 TTAATATTTT TGAGGAGCCT GCAACATGCC AGCCACTGTG ATAGAGGCTG GCGGATCCAA 780
 15 GCAAATGGCC AATGAGATCA TTGTGAAGGC AGGGGAATGT ATGTGCACAT CTGTTTTGTA 840
 ACTGTTTAGA TGAATGTCAG TTGTATTTTA TTGAAATGAT TTCACAGTGT GTGGTCAACA 900
 TTTCTCATGT TGAAACTTTA AGAACTAAAA TGTTCTAAAT ATCCCTTGGA CATTTTATGT 960
 20 CTTTCTTGTA AGGCATACTG CCTTGTTTAA TGGTAGTTT ACAGTGTTTC TGGCTTAGAA 1020
 CAAAGGGGCT TAATTATTGA TGTTTTCGGA 1050

25 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Ala Arg Ala Thr Leu Ser Ala Ala Pro Ser Asn Pro Arg Leu Leu
 1 5 10 15
 Arg Val Ala Leu Leu Leu Leu Leu Val Ala Ala Ser Arg Arg Ala
 20 25 30
 Ala Gly Ala Pro Lys Ala Thr Glu Lys Arg Cys Gln Cys Lys Gln Thr
 35 40 45
 Leu Gln Gly Ile His Leu Lys Asn Ile Gln Ser Val Lys Val Lys Ser
 50 55 60
 Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys Asn
 65 70 75 80
 Gly Gln Lys Ala Cys Leu Asn Pro Ala Ser Pro Met Val Lys Lys Ile
 85 90 95
 Ile Glu Lys Met Leu Lys
 100

60 (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1081 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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CTCTCCTCCT CGCACAGCCG CTCGAACCGC CTGCTGAGCC CCATGGCCCG CGCCACGCTC      60
5  TCCGCCGCCC CCAGCAATCC CCGGCTCCTG CGGGTGGCGC TGCTGCTCCT GCTCCTGGTG      120
   GCCGCCAGCC GGCGCGCAGC AGGAGCGCCC CTGGCCACTG AACTGCGCTG CCAGTGCTTG      180
   CAGACCCTGC AGGGAATTCA CCTCAAGAAC ATCCAAAGTG TGAAGGTGAA GTCCCCCGGA      240
10  CCCCCTGCG CCCAAACCGA AGTCATAGCC AACTCAAGA ATGGGCAGAA AGCTTGCTC      300
   AACCCGCGAT CGCCCATGGT TAAGAAAATC ATCGAAAAGA TGCTGAAAAA TGGCAAATCC      360
15  AACTGACCAG AAGGAAGGAG GAAGCTTATT GGTGGCTGTT CCTGAAGGAG GCCCTGCCCT      420
   TACAGGAACA GAAGAGGAAA GAGAGACACA GCTGCAGAGG CCACCTGGAT TGCGCCTAAT      480
   GTGTTTGAGC ATCACTTAGG AGAAGTCTTC TATTTATTTA TTTATTTATT TATTGTTTG      540
20  TTTTAGAAGA TTCTATGTTA ATATTTTATG TGTAATAATA GGTTATGATT GAATCTACTT      600
   GCACACTCTC CCATTATATT TATTGTTTAT TTTAGGTCAA ACCCAAGTTA GTTCAATCCT      660
25  GATTCAATATT TAATTTGAAG ATAGAAGGTT TGCAGATATT CTCTAGTCAT TTGTTAATAT      720
   TTCTTCGTGA TGACATATCA CATGTCAGCC ACTGTGATAG AGGCTGAGGA ATCCAAGAAA      780
   ATGGCCAGTG AGATCAATGT GACGGCAGGG AAATGTATGT GTGTCTATTT TGTAAGTGTA      840
30  AAGATGAATG TCAGTTGTTA TTTATTGAAA TGATTTCACA GTGTGTGGTC AACATTTCTC      900
   ATGTTGAAGC TTTAAGAACT AAAATGTTCT AAATATCCCT TGGACATTTT ATGCTTTTCT      960
35  TGTAAGGCAT ACTGCCTTGT TTAATGTTAA TTATGCAGTG TTCCCTCTG TGTTAGAGCA      1020
   GAGAGGTTTC GATATTTATT GATGTTTCA CAAAGAACAG GAAAATAAAA TATTTAAAAA      1080
   T                                                                                   1081
40

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(2) INFORMATION FOR SEQ ID NO:20:

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(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 107 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

55  Met Ala His Ala Thr Leu Ser Ala Ala Pro Ser Asn Pro Arg Leu Leu
    1           5           10           15
    Arg Val Ala Leu Leu Leu Leu Leu Val Ala Ala Ser Arg Arg Ala
        20           25           30
    Ala Gly Ala Ser Val Val Thr Glu Leu Arg Cys Gln Cys Leu Gln Thr
        35           40           45
    Leu Gln Gly Ile His Leu Lys Asn Ile Gln Ser Val Asn Val Arg Ser
        50           55           60
    Pro Gly Pro His Cys Ala Gln Thr Glu Val Ile Ala Thr Leu Lys Asn
        65           70           75           80

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Gly Lys Lys Ala Cys Leu Asn Pro Ala Ser Pro Met Val Gln Lys Ile
85 90 95

Ile Glu Lys Ile Leu Asn Lys Gly Ser Thr Asn
100 105

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 988 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTCGCACAGC	TTCCCCGACGC	GTCTGCTGAG	CCCCATGGCC	CACGCCACGC	TCTCCGCGCG	60
CCCCAGCAAT	CCCCGGCTCC	TGCGGGTGGC	GCTGCTGCTC	CTGCTCCTGG	TGGCCGCCAG	120
CCGGCGCGCA	GCAGGAGCGT	CCGTGGTCAC	TGAAGTGGCG	TGCCAGTGCT	TGCAGACACT	180
GCAGGGAATT	CACCTCAAGA	ACATCCAAAG	TGTGAATGTA	AGGTCCCCCG	GACCCCACTG	240
CGCCCAAACC	GAAGTCATAG	CCACACTCAA	GAATGGGAAG	AAAGCTTGTC	TCAACCCCGC	300
ATCCCCCATG	GTTCAGAAAA	TCATCGAAAA	GATACTGAAC	AAGGGGAGCA	CCAAC TGACA	360
GGAGAGAAGT	AAGAAGCTTA	TCAGCGTATC	ATTGACACTT	CCTGCAGGGT	GGTCCCTGCC	420
CTTACCAGAG	CTGAAAAATGA	AAAAGAGAAO	AGCAGCTTTC	TAGGGACAGC	TGGAAAGGAO	480
TTAATGTGTT	TGACTATTTT	TTACGAGGGT	TCTACTTATT	TATGTATTTA	TTTTTTGAAAG	540
CTTGTATTTT	AATATTTTAC	ATGCTGTTAT	TTAAAGATGT	GAGTGTGTTT	CATCAAACAT	600
AGCTCAGTCC	TGATTATTTA	ATTGGAATAT	GATGGGTTTT	AAATGTGTCA	TTAAACTAAT	660
ATTTAGTGGG	AGACCATAAT	GTGTCAGCCA	CCTTGATAAA	TGACAGGGTG	GGGAAGTGGA	720
GGGTGGGGGG	ATTGAAATGC	AAGCAATTAG	TGGATCACTG	TTAGGGTAAG	GGAATGTATG	780
TACACATCTA	TTTTTTTATAC	TTTTTTTTTTA	AAAAAAGAAT	GTCAGTTGTT	ATTTATTCAA	840
ATTATCTCAC	ATTATGTGTT	CAACATTTTT	ATGCTGAAGT	TTCCCTTAGA	CATTTTATGT	900
CTTGCTTGTA	GGGCATAATG	CCTTGTTTAA	TGTCCATTCT	GCAGCGTTTC	TCTTTCCTT	960
GGAAAAGAGA	ATTTATCATT	ACTGTTAC				988

(2) INFORMATION FOR SEQ ID NO:22:

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 97 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Thr Ser Lys Leu Ala Val Ala Leu Leu Ala Ala Phe Leu Ile Ser
1 5 10 15

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Ala Ala Leu Cys Glu Gly Ala Val Leu Pro Arg Ser Ala Lys Glu Leu
 20 25 30
 5 Arg Cys Gln Cys Ile Lys Thr Tyr Ser Lys Pro Phe His Pro Lys Phe
 35 40 45
 Ile Lys Glu Leu Arg Val Ile Glu Ser Gly Pro His Cys Ala Asn Thr
 50 55 60
 10 Glu Ile Ile Val Lys Leu Ser Asp Gly Arg Glu Leu Cys Leu Asp Pro
 65 70 75 80
 Lys Glu Asn Trp Val Gln Arg Val Val Glu Lys Phe Leu Lys Arg Ala
 85 90 95
 15 Glu

(2) INFORMATION FOR SEQ ID NO:23:

- 20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 291 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 25 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

30 ATGACTTCCA AGCTGGCCGT GGCTCTCTTG GCAGCCTTCC TGATTTCTGC AGCTCTGTGT 60
 GAAGGTGCAG TTTTGCCAAG GAGTGCTAAA GAACTTAGAT GTCAGTGCAT AAAGACATAC 120
 35 TCCAAACCTT TCCACCCCAA ATTTATCAAA GAACTGAGAG TGATTGAGAG TGGACCACAC 180
 TCGGCCAACA CAGAAATTAT TGTAAGCTT TCTGATGGAA GAGAGCTCTG TCTGGACCCC 240
 40 AAGGAAACT GGGTCAGAG GGTGTGGAG AAGTTTTGA AGAGGGCTGA G 291

(2) INFORMATION FOR SEQ ID NO:24:

- 45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 78 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

55 Ala Gly Pro Ala Ala Ala Val Leu Arg Glu Lys Arg Cys Val Cys Leu
 1 5 10 15
 Gln Thr Thr Gln Gly Val His Pro Lys Met Ile Ser Asn Leu Gln Val
 20 25 30
 60 Phe Ala Ile Gly Pro Gln Cys Ser Lys Val Glu Val Val Ala Ser Leu
 35 40 45
 Lys Asn Gly Lys Glu Ile Cys Leu Asp Pro Glu Ala Pro Phe Leu Lys
 50 55 60
 65 Lys Val Ile Gln Lys Ile Leu Asp Gly Gly Asn Lys Glu Asn
 65 70 75

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(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 216 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GTGTTGCGGG AACTGCGGTG CGTGTGTTTA CAGACCACGC AGGGAGTTCA TCCCAAAATG 60
 15 ATCAGTAATC TGCAAGTGTT CGCCATAGGC CCACAGTGCT CCAAGGTGGA AGTGGTAGCC 120
 TCCCTGAAGA ACGGGAAGGA AATTGTCTT GATCCAGAAG CCCCTTTTCT AAAGAAAGTC 180
 ATCCAGAAAA TCCTCGACGG CGGCAACAAA GAAAAAC 216

20

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 93 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: peptide

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

35 Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
 1 5 10 15
 Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
 20 25 30
 40 Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
 35 40 45
 45 Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
 50 55 60
 Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
 65 70 75 80
 50 Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala
 85 90

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4788 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

65 GAATTCAAGG CCTGTCCTGG TTTGGTCCCA ATTTACCTTT ATCATCCATA TTCACCCCA 60
 CTGCTCTGCA GCTCCACTGA AGCACCCCTT CTTTCTCTG AGCCACAATG TCACCCAG 120
 GACTCTGCCT CAGCTGGGCC TCCACTGCCC ACCCATCTAT AGATGCCTAA ATCCCGGGCA 180

	GTTATCCAGA	CACAACTAAA	GTTCCATCCC	TTCCATGAAG	CCTTCCCCAA	CCCTCTGGTG	240
	GAAGGTCACT	TCTTCCTCAT	GGGGTTCTGA	GCTTTTCATT	CTTTTCTAC	TAAGAGTTTT	300
5	ACAATTACCT	GTTCATACAC	TCTACCTGCC	CCCATGAGAC	CAGGGGCATC	TCAGAAACAA	360
	AGATCATTAA	AACCAACTAA	ATCTATTTCT	CATTATAAAA	TGAGATATGC	TGATTGATTG	420
	CAAAATAATA	AAATAACAAA	GTATGGAAAA	GAAAAAATAA	AGCATATAAT	CTGGCTGAGA	480
10	AGGTAGAGAC	CCTTCCACAC	CACTGAAATT	ATGTGTTGAA	AAGAATAAGG	AAAAAAGTGC	540
	TTCAGTTTGG	CATTATTTAT	GTAAGTATAG	TATAGGATCC	TTAAAATGGT	TCAAAGAAAT	600
15	GGGAAATCAA	GACTTCATTT	TGGCAAAGCC	ATTGAACAGA	AACTGTAGCA	TATTTATCAG	660
	TAATTTCTTT	CAGATTAAAC	AACTGACAAC	AACCCACTTT	TCAACCAGTG	ATGTTGGAAA	720
	TGTTTTAAAA	CAAAATTAGT	TCATAAATTT	GTGGGTTGAC	CAAGAAGGTA	ATAAAGTCTC	780
20	ACTAAATAAA	ATGAGGAAAA	TTCAGAAAAA	GAAAAAATAA	AGAAAATAAA	TCACCCATGG	840
	ATCTAAGCAC	TATTCATTCT	TTAAGGCATG	TATTTCCAAG	CCTTTTAATT	TTTTCATGCC	900
25	TAGAGTTGGC	ATGGCATATA	TATATCTTTA	TACAATTCTT	CAAATTTTAT	AGAATTTGTA	960
	TAATGTTTTA	TCTTGCTTTT	TTTTTAACCA	CTGATGTTAT	AAGCATATTT	ATGCCACTTC	1020
	ATTCACGTTA	GAGACTTAAT	AATAAAGGAT	CTTGTGGATA	ATTTATCATT	CCCTGATAGA	1080
30	GAAAAATTTA	GCTTTGCTTA	TTTTAGAGTT	ATAAATGATG	CTGGGTCAGG	TATCTTTATG	1140
	TTTGAAGATG	GCTCCATATT	TGGGTTGTTT	CCACAGAACT	CTTCCAGAA	ATGCTTTTTC	1200
35	TAGGTTAATG	GCTACACATA	TTTCTAGGCA	CCTGACATAC	TGACACCCAC	CTCTAAAGTA	1260
	TTTTTATGAT	CCACAAC TAG	CGTTTAACAC	AGCGCCCCAG	TCACTCCGAG	ACTAATAAAT	1320
	AGACAAATGA	CTGAAACGTG	ACCTCATGCT	TTCTATTCCG	CCAGCTTTCA	TTGAGTTCCT	1380
40	TTCTCTGGG	AGGACTGGGG	GTTGTCTAGC	CCTCCACAGC	ATCAGCCCAT	TGACCCTATC	1440
	CTTGTGGTTA	TAGCAGCTGA	GGAAGCAGAA	TTACAGCTCT	GTGGGAAGGA	ATGGGGCTGG	1500
45	AGAGTTCATG	CATAGACCAA	TTCTTTTTTT	TTTTTTTTTT	TGAGATGGAG	TTTCACTTTT	1560
	GTTGCCCAGG	CTGGAGTGCA	ATGGCATGAT	CTCAGCTCAC	CACAGCCCCC	ACCTCCTGGG	1620
	TTCAAGCGAT	TCTCCTGCCC	TCAGCCTCCC	GAGTAGCTGG	GATTACAGGC	ATGTGCCACC	1680
50	ACGCCTGACT	ACTTTTGTAT	TTTTAGTAGA	GATGGAGTTT	CTCTTTCTTG	GTCAGGTTGG	1740
	TCTCAAACCTC	CTGACCTCAG	GTGATCTGCA	GCCTCGGCCT	CCAAAGTGTT	GGGATTACAG	1800
55	GTGTGAGCGA	CCATGCCTGG	CTGCATAGAC	CAGTTCTTAT	GAGAAGGGAT	CAACTAAGAA	1860
	TAGCCTTGGG	TTGACACACA	CCCCTCTTCA	CACTCACAGG	AGAAACCCCA	TGAAGCTAGA	1920
	ACCAGTCATG	AGTTGAGAGC	TGAGAGTTAG	AGAGTAGCTC	AGAGATGCTA	TTCTTGAGTA	1980
60	TCCTGAGCCC	CTGTGGTCA	CAGGGACCC	GAGTTGTGCA	AACTCAGCA	TGACAGCATC	2040
	ACTACACTTA	AAAATTTCCC	TCCTCACCCC	CAGATTCCAT	TTCCCCATCC	GCCAGGGCTG	2100
65	CCTATAAAGA	GGAGAGATGG	CTTCAGACAT	CAGAAGGACG	CAGGCAGCAA	AGAGTAGTCA	2160
	GTCCCTTCTT	GGCTCTGCTG	ACACTCGAGC	CCACATTCCA	TCACCTGCTC	CCAATCATGC	2220
	AGGTCCTCAC	TGCTGCCCTT	GCCGTCCTCC	TCTGCACCAT	GGCTCTCTGC	AACCAGGTCC	2280

	TCTCTGCACC	ACGTGAGTCC	ATGTTGTTGT	TGTGGGTATC	ACCACTCTCT	GGCCATGGTT	2340
	AGACCACATC	AGTCTTTTTT	TGTGGCGTGA	GAGGCCCCGA	AGAGAAAAGA	AGGAAGTTCT	2400
5	TAAAGCGCTG	CCAAACACCT	TGGTCTTTTT	CTTCACAAC	TTTATTTT	TCTCTAGAAG	2460
	GGGTCTTAGC	CCTCCTAGTC	TCCAGGTATG	AGAATCTAGG	CAGGGGCAGG	GGAGTTACAG	2520
	TCCCTTGATC	AGATAGAAAA	ACAGGGTTCA	AAACGAATCA	GTTTGCAAGA	GGCAGAATCC	2580
10	AGGGCTGCTT	ACTTCCCAGT	GGGGTCTGTT	CTTCACTCTC	CAGCTCACCC	TAGTCTCCCA	2640
	GGAGCCCTGT	CCCTTGATG	TCTTATGAGA	GATGTCCAGG	GCTTCTCTTG	GGCTGGGGTA	2700
15	TGACTTCTTG	AACCGACAAA	ATTCCATGAA	GAGAGCTAAG	AGAACAGTCC	ATTCAGGTAT	2760
	CTGGATCACA	TAGAGAAACA	GAGAACCCAC	TATGAAGAGT	CAAGGGGAAA	GAGGAATATA	2820
	GACAGAAACA	AAGAGACATT	TCTCTGCAAA	ACCCCCAAA	TGCCTTGAG	TCACTTGGTC	2880
20	TGAGCAAGCC	TGCCCTCCTC	AACCACTCAG	GGATCAGAAG	CTGCCCTGGC	TTTCTTCTG	2940
	AGCTGTGACT	TGGGCTTATT	CTCTCCTTTC	TCCGCAGTTG	CTGCTGACAC	GCCGACCGCC	3000
25	TGCTGCTTCA	GCTACACCTC	CCGACAGATT	CCACAGAATT	TCATAGCTGA	CTACTTTGAG	3060
	ACGAGCAGCC	AGTGCTCCAA	GCCCAGTGTC	ATGTAAGTGC	CAGTCTTCCT	GCTCACCTCT	3120
	AGGGAGGTAG	GGAGTGTGAG	GGTGGGGGCA	GAAACAGGCC	AGAAGGCCAT	CCTGGAAAGG	3180
30	CCCAGCCTTC	AGGAGCCTAT	CGGGGATACA	GGACGCAGGG	CACTGAGGTG	TGACCTGACT	3240
	TGGGGCTGGA	GTGAGGTGGG	TGTTACAGAG	TCAGGAAGGG	CTGCCCCAGG	CCAGAGGAAA	3300
35	GGGACAGGAA	GAAGGAGGCA	GCAGGACACT	CTGAGGGCCC	CCTTGCCCTGG	AGTCACTGAG	3360
	AGAAGCTCTC	TAGACGGAGA	TAGGCAGGGG	GCCCCTGAGA	GAGGAGCAGG	CCTTGAGCTG	3420
	CCCAGGACAG	AGAGCAGGAT	GTCAGGGCCA	TGGTGGGCCC	AGGATTCCCC	GGCTGGATTG	3480
40	CCCAGTGCTT	AACCTTCTCT	CCCTTCTCCA	CAGCTTCTTA	ACCAAGAGAG	GCCGGCAGGT	3540
	CTGTGCTGAC	CCCAGTGAGG	AGTGGGTCCA	GAAATACGTC	AGTGACCTGG	AGCTGAGTGC	3600
45	CTGAGGGGTC	CAGAAGCTTC	GAGGCCCAGC	GACCTCAGTG	GGCCAGTGG	GGAGGAGCAG	3660
	GAGCCTGAGC	CTTGGGAACA	TGCGTGTGAC	CTCCACAGCT	ACCTCTTCTA	TGGACTGGTT	3720
	ATTGCCAAAC	AGCCACACTG	TGGGACTCTT	CTTAACCTAA	ATTTTAATTT	ATTTATACTA	3780
50	TTTAGTTTTT	ATAATTTATT	TTTGATTTC	CAGTGTGTTT	GTGATTGTTT	GCTCTGAGAG	3840
	TTCCCCCTGT	CCCCTCCACC	TTCCCTCACA	GTGTGTCTGG	TGACAACCGA	GTGGCTGTCA	3900
55	TCGGCCTGTG	TAGGCAGTCA	TGGCACCAAA	GCCACCAGAC	TGACAAATGT	GTATCAGATG	3960
	CTTTTGTTCA	GGGCTGTGAT	CGGCCTGGGG	AAATAATAAA	GATGTTCTTT	TAAACGGTAA	4020
	ACCAATATTG	AGTTTGGTTT	TGTTTTTCTG	GCAATCAAAA	ATCACTGGTT	AAGAGGAATC	4080
60	ATAGGCAAAG	ATTAGGAAGA	GGTGAAATGG	AGGGAAATGG	GGAGAGATGG	GGAGCGCTGC	4140
	GACAGAGTTA	TCCACTTCAC	AAAATTCTGG	AACATTGAAA	CTACGAATAT	GTTATAACTC	4200
65	AAATCGTAAT	ATGCACGCTC	TAGGAGAATT	AACTACTTGA	ATGGCCACCA	TTAAGCAGAG	4260
	TATTCTGTAG	GGCATATTCA	TGATGAATCA	AGCTCTTAAT	AGCAATTATT	TACATTGTTG	4320
	AGGCTTACTC	CTCCTACTGA	GTGCTTTTTA	TACATTGTTC	ATTTAATCTT	ACCAATGCAA	4380

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TAGTACAGCT TAGGTACTAT TAATACCTCC ACTTGACAGA AAAGTAACCC AGGGCTCAGA 4440
 AAGGTTAGAC AACTTGGCTG AGGTTACACA GCACGTAAAC GGTCAATTGT GTTCCAAAAC 4500
 5 TGGACTTTTA TTGAACTACA GACTATGCTG TTAACCATTG ACCAAGTTAT TTCCCAAAGT 4560
 ATGACCCGCC TATACTCAAA TCTTACCCCA TTCTTTAACA GATGATACTT TATCCATTGC 4620
 AACCCTTCC TGTCAGGATT CTGAGTTGAC ATAGAGTGTT TCAGCAGTGA TTATTTAAGC 4680
 10 CAATTACATC AGGATCTTTA GGTGTAGACC TGGGAACTGA TATTTTATC AAGCTCATGA 4740
 GGTGTTCCAT AGCATGTAA TGACTGAGAG CCACTGTCAA TAGAATTC 4788

15 (2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 92 amino acids
 (B) TYPE: amino acid
 20 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Lys Leu Cys Val Thr Val Leu Ser Leu Leu Met Leu Val Ala Ala
 1 5 10 15
 Phe Cys Ser Pro Ala Leu Ser Ala Pro Met Gly Ser Asp Pro Pro Thr
 20 25 30
 35 Ala Cys Cys Phe Ser Tyr Thr Ala Arg Lys Leu Pro Arg Asn Phe Val
 35 40 45
 Val Asp Tyr Tyr Glu Thr Ser Ser Leu Cys Ser Gln Pro Ala Val Val
 50 55 60
 40 Phe Gln Thr Lys Arg Ser Lys Gln Val Cys Ala Asp Pro Ser Glu Ser
 65 70 75 80
 45 Trp Val Gln Glu Tyr Val Tyr Asp Leu Glu Leu Asn
 85 90

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 696 base pairs
 50 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTCCCCCCCC CCCCCCCCCC CCCC GCCGACA GCACAGGACA CAGCTGGGTT CTGAAGCTTC 60
 60 TGAGTTCTGC AGCCTCACCT CTGAGAAAAC CTCTTTTCCA CCAATACCAT GAAGCTCTGC 120
 GTGACTGTCC TGTCTCTCCT CATGCTAGTA GCTGCCTTCT GCTCTCCAGC GCTCTCAGCA 180
 65 CCAATGGGCT CAGACCCTCC CACCGCCTGC TGCTTTTCTT ACACCGCGAG GAAGCTTCCT 240
 CGCAACTTTG TGGTAGATTA CTATGAGACC AGCAGCCTCT GCTCCCAGCC AGCTGTGGTA 300
 TTCCAAACCA AAAGAAGCAA GCAAGTCTGT GCTGATCCCA GTGAATCCTG GGTCCAGGAG 360

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GGGCTCTGGA AACCACATGG CTTACCTGT CCCCAGAACT ACCAGCCCTA CACCATTCTT 420
 TCTGCCCTGC TTTTGCTAGG TCACAGAGGA TCTGCTTGGT CTTGATAAGC TATGTTGTTG 480
 5 CACTTTAAAC ATTTAAATTA TACAATCATC AACCCCCAAC 520

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
 10 (A) LENGTH: 99 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

20 Met Lys Val Ser Ala Ala Leu Leu Cys Leu Leu Leu Ile Ala Ala Thr
 1 5 10 15
 25 Phe Ile Pro Gln Gly Lys Ala Gln Pro Asp Ala Ile Asn Ala Pro Val
 20 25 30
 Thr Cys Cys Tyr Asn Phe Thr Asn Arg Lys Ile Ser Val Gln Arg Leu
 35 40 45
 30 Ala Ser Tyr Arg Arg Ile Thr Ser Ser Lys Cys Pro Lys Glu Ala Val
 50 55 60
 Ile Phe Lys Thr Ile Val Ala Lys Glu Ile Cys Ala Asp Pro Lys Gln
 65 70 75 80
 35 Lys Trp Val Gln Asp Ser Met Asp His Leu Asp Lys Gln Thr Gln Thr
 85 90 95
 40 Pro Lys Thr

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
 45 (A) LENGTH: 725 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CTAACCAGAG AACATCCAAT TCTCAAACTG AAGCTCGCAC TCTCGCCTCC AGCATGAAAG 60
 55 TCTCTGCCGC CTTTCTGTGC CTGCTGCTCA TAGCAGCCAC CTTTATTTCC CAAGGGCTCG 120
 CTCAGCCAGA TGCAATCAAT GCCCCAGTCA CTTGCTGTGA TAACCTCAAC AATAGGAAGA 180
 60 TCTCAGTGCA GAGGCTCGCG AGCTATAGAA GAATCACCAG CAGCAAGTGT CCCAAAGAAG 240
 CTGTGATCTT CAAGACCATT GTGGCCAAGG AGATCTGTGC TGACCCCAAG CAGAAGTGGG 300
 TTCAGGATTC CATGGACCAC CTGGACAAGC AAACCCAAAC TCCGAAGACT TGAACACTCA 360
 65 CTCACAACC CAAGAATCTG CAGCTAACTT ATTTTCCCT AGCTTTCCC AGACACCCTG 420
 TTTTATTTTA TTATAATGAA TTTTGTGTGT TGATGTGAAA CATTATGCCT TAAGTAATGT 480

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TAATTCTTAT TTAAGTTATT GATGTTTAA GTTATCTTT CATGGTACTA GTGTTTTTTA 540
 GATACAGAGA CTTGGGGAAA TTGCTTTTCC TCTTGAACCA CAGTTCTACC CCTGGGATGT 600
 5 TTTGAGGGTC TTTGCAAGAA TCATTAATAC AAAGAATTTT TTTTAACATT CCAATGCATT 660
 GCTAAATAT TATTGTGGAA ATGAATATTT TGTAACTATT ACACCAAATA AATATATTTT 720
 TGTAC 725

10 (2) INFORMATION FOR SEQ ID NO:34:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 99 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

25 Met Lys Ala Ser Ala Ala Leu Leu Cys Leu Leu Leu Thr Ala Ala Ala
 1 5 10 15
 Phe Ser Pro Gln Gly Leu Ala Gln Pro Val Gly Ile Asn Thr Ser Thr
 20 25 30
 30 Thr Cys Cys Tyr Arg Phe Ile Asn Lys Lys Ile Pro Lys Gln Arg Leu
 35 40 45
 Glu Ser Tyr Arg Arg Thr Thr Ser Ser His Cys Pro Arg Glu Ala Val
 50 55 60
 Ile Phe Lys Thr Lys Leu Asp Lys Glu Ile Cys Ala Asp Pro Thr Gln
 65 70 75 80
 40 Lys Trp Val Gln Asp Phe Met Lys His Leu Asp Lys Lys Thr Gln Thr
 85 90 95
 Pro Lys Leu

45 (2) INFORMATION FOR SEQ ID NO:35:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 810 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AGCAGAGGGG CTGAGACCAA ACCAGAAACC TCCAATTCTC ATGTGGAAGC CCATGCCCTC 60
 60 ACCCTCCAAC ATGAAAGCCT CTGCAGCACT TCTGTGTCTG CTGCTCACAG CAGCTGCTTT 120
 CAGCCCCCAG GGGCTTGCTC AGCCAGTTGG GATTAATACT TCAACTACCT GCTGCTACAG 180
 ATTTATCAAT AAGAAAATCC CTAAGCAGAG GCTGGAGAGC TACAGAAGGA CCACCAGTAG 240
 65 CCACTGTCCC CCGGAAGCTG TAATCTTCAA GACCAAACTG GACAAGGAGA TCTGTGCTGA 300
 CCCCACACAG AAGTGGGTCC AGGACTTTAT GAAGCACCTG GACAAGAAAA CCCAAACTCC 360

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AAAGCTTTGA ACATTCATGA CTGAACTAAA AACAAAGCCAT GACTTGAGAA ACAAATAATT 420
 TGTATACCCT GTCCTTTCTC AGAGTGGTTC TGAGATTATT TTAATCTAAT TCTAAGGAAT 480
 5 ATGAGCTTTA TGTAAATAATG TGAATCATGG TTTTCTTAG TAGATTTTAA AAGTTATTAA 540
 TATTTTAATT TAATCTTCCA TGGATTTTGG TGGGTTTGA ACATAAAGCC TTGGATGTAT 600
 ATGTCATCTC AGTGCTGTAA AAAGTGTGGG ATGCTCCTCC CTTCTCTACC TCATGGGGGT 660
 10 ATTGATAAAG TCCTTGCAAG AATCAGTGCA AAGATTTGCT TTAATTGTTA AGATATGATG 720
 TCCCTATGGA AGCATATTGT TATTATATAA TTACATATTT GCATATGTAT GACTCCCAA 780
 15 TTTTCACATA AAATAGATT TTGTAAAAA 810

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
 20 (A) LENGTH: 91 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Lys Val Ser Ala Ala Arg Leu Ala Val Ile Leu Ile Ala Thr Ala
 1 5 10 15
 Leu Cys Ala Pro Ala Ser Ala Ser Pro Tyr Ser Ser Asp Thr Thr Pro
 20 25 30
 Cys Cys Phe Ala Tyr Ile Ala Arg Pro Leu Pro Arg Ala His Ile Lys
 35 40 45
 Glu Tyr Phe Tyr Thr Ser Gly Lys Cys Ser Asn Pro Ala Val Val Phe
 50 55 60
 Val Thr Arg Lys Asn Arg Gln Val Cys Ala Asn Pro Glu Lys Lys Trp
 65 70 75 80
 Val Arg Glu Tyr Ile Asn Ser Leu Glu Met Ser
 85 90

(2) INFORMATION FOR SEQ ID NO:37:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1160 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 55 (D) TOPOLOGY: linear

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CCTCCGACAG CCTCTCCACA GGTACCATGA AGGTCTCCGC GGCACGCCTC GCTGTATCC 60
 TCATTGCTAC TGCCCTCTGC GCTCCTGCAT CTGCCCTCCC ATATTCCTCG GACACCACAC 120
 65 CCTGCTGCTT TGCCTACATT GCGCGCCAC TGCCCGTGC CCACATCAAG GAGTATTTCT 180
 ACACCAAGTGG CAAGTGCTCC AACCCAGCAG TCGTCTTTGT CACCCGAAAG AACCGCCAAG 240
 TGTGTGCCAA CCCAGAGAAG AAATGGGTTC GGGAGTACAT CAACTCTTTG GAGATGAGCT 300

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AGGATGGAGA GTCCTTGAAC CTGAACCTTAC ACAAATTGTC CTGTTTCTGC TTGCTCTTGT 360
 CCTAGCTTGG GAGGCTTCCC CTCACTATCC TACCCCACCC GCTCCTTGAA GGGCCCAGAT 420
 5 TCTGACCACG ACGAGCAGCA GTTACAAAAA CCTTCCCCAG GCTGGACGTG GTGGCTCAGC 480
 CTTGTAATCC CAGCACTTTG GGAGGCCAAG GTGGGTGGAT CACTTGAGGT CAGGAGTTCG 540
 AGACAGCCTG GCCAACATGA TGAAACCCCA TGTGTACTAA AAATACAAA AATTAGCCGG 600
 10 GCGTGGTAGC GGGCGCCTGT AGTCCCAGCT ACTCGGGAGG CTGAGGCAGG AGAATGGCGT 660
 GAACCCGGGA GCGGAGCTTG CAGTGAGCCG AGATCGCGCC ACTGCACTCC AGCCTGGGCG 720
 15 ACAGAGCGAG ACTCCGTCTC AAAAAAAAAA AAAAAAAAAA AAAAAATACA AAAATTAGCC 780
 GCGTGGTGGC CCACGCCTGT AATCCCAGCT ACTCGGGAGG CTAAGGCAGG AAAATTGTTT 840
 GAACCCAGGA GGTGGAGGCT GCAGTGAGCT GAGATTGTGC CACTTCACTC CAGCCTGGGT 900
 20 GACAAAGTGA GACTCCGTCA CAACAACAAC AACAAAAAGC TTCCCAACT AAAGCCTAGA 960
 AGAGCTTCTG AGGCGCTGCT TTGTCAAAG GAAGTCTCTA GGTTCTGAGC TCTGGCTTTG 1020
 25 CCTTGGCTTT GCAAGGGCTC TGTGACAAGG AAGGAAGTCA GCATGCCTCT AGAGGCAAGG 1080
 AAGGGAGGAA CACTGCACTC TTAAGCTTCC GCCGTCTCAA CCCCTCACAG GAGCTTACTG 1140
 GCAAACATGA AAAATCGGGG 1160

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 97 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

45 Met Arg Ile Ser Ala Thr Leu Leu Cys Leu Leu Leu Ile Ala Ala Ala
 1 5 10 15
 Phe Ser Ile Gln Val Trp Ala Gln Pro Asp Gly Pro Asn Ala Ser Thr
 20 25 30
 50 Cys Cys Tyr Val Lys Lys Gln Lys Ile Pro Lys Arg Asn Leu Lys Ser
 35 40 45
 55 Tyr Arg Arg Ile Thr Ser Ser Arg Cys Pro Trp Glu Ala Val Ile Phe
 50 55 60
 Lys Thr Lys Lys Gly Met Glu Val Cys Arg Glu Ala His Gln Lys Trp
 65 70 75 80
 60 Val Glu Glu Ala Ile Ala Tyr Leu Asp Met Lys Thr Pro Thr Pro Lys
 85 90 95
 Pro

65

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(2) INFORMATION FOR SEQ ID NO:39:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 593 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

ACTGAAGCCA GCTCTCTCAC TCTCTTCTC CACCATGAGG ATCTCTGCCA CGCTTCTGTG 60
 15 CCTGCTGCTC ATAGCCGCTG CTTTCAGCAT CCAAGTGTGG GCCCAACCAG ATGGGCCCAA 120
 TGCATCCACA TGCTGCTATG TCAAGAAAC AAGATCCCC AAGAGGAATC TCAAGAGCTA 180
 DO CAGAAGGATC ACCAGTAGTC GGTGTCCCTG GGAAGCTGTT ATCTTCAAGA CAAAGAAGGG 240
 20 CATGGAAGTC TCTCGTGAAG CCCATCAGAA GTGGGTCGAG GAGGCTATAG CATACTTAGA 300
 CATGAAAACC CCAACTCCAA AGCCTTGAAG AAATGTGCCT GAACAGAAAC CAACCTAGGA 360
 25 GCCAAGAAGC AAAAATTCCT CACCGCTGTT CTTTCTGAGA ACTGTTGATG AAATGTGTTG 420
 ATCACGGTCC TAAGGGATAG GAGCTGTCTG TAGGAATGTG AAACAGTCAC GCCTAAGGAA 480
 TGGTCTTTAA GTTATTAATA TTTTATTATA ATTAGCCATG TACTTTGGTG TGATTTGAAT 540
 30 GTAAAGCTCT GGAGACCTCA TGCACTTTA ACATTGTGTT AGCTGCAGAA TTC 593

(2) INFORMATION FOR SEQ ID NO:40:

- 35 (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 72 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: peptide

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Asp Ser Val Ser Ile Phe Ile Thr Cys Cys Phe Asn Val Ile Asn Arg
 1 5 10 15
 50 Lys Ile Pro Ile Gln Arg Leu Glu Ser Tyr Thr Arg Ile Thr Asn Ile
 20 25 30
 Gln Cys Pro Lys Glu Ala Val Ile Phe Lys Thr Gly Lys Glu Val Cys
 35 40 45
 55 Ala Asp Pro Lys Glu Arg Trp Val Arg Asp Ser Met Lys His Lys Asp
 50 55 60
 60 Gln Ile Phe Gln Asn Leu Lys Pro
 65 70

(2) INFORMATION FOR SEQ ID NO:41:

- 65 (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Asn Leu Ala Lys Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Cys
1 5 10 15

10 (2) INFORMATION FOR SEQ ID NO:42:

(1) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

25 Cys Asn Gln Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys Ile
1 5 10 15

Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys
20 25 30

30 (2) INFORMATION FOR SEQ ID NO:43:

(1) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TGCAACCAAG TCGAAGTGAT AGCCACACTG AAGGATGGGA GGAAAATCTG CCTGGACCCA 60

45 GATGCTCCCA GAATCAAGAA AATTGTACAG AAAAAA 96

CLAIMS

We claim:

1. A method of screening for AHA compounds comprising the steps of:
 - a) contacting a compound with radiolabeled heparin/heparan sulfate and heparanase;
 - b) maintaining the compounds in contact with the radiolabeled heparin/heparan sulfate and heparanase for a time and under such conditions sufficient to allow inhibition of heparanase activity;
 - c) detecting inhibition of heparanase activity (a compound that gives 50% inhibition at a concentration of 1 μ M or less); and
 - d) selecting compounds that inhibit heparanase activity.
2. A method according to claim 1 wherein the heparanase is recombinant.
3. A heparanase having an isoelectric point of less than 5.5 and possessing activity greater than 20 units heparanase activity per μ g protein.
4. A heparanase according to Claim 3, having an isoelectric point of about 4.8 - 5.1.
5. A heparanase purified to apparent homogeneity, as in claim 3, prepared in the presence of reducing conditions and activated with transglutaminase, having an amino acid sequence selected from the group consisting of SEQ. ID NO: 1, SEQ. ID NO: 3, SEQ. ID NO: 5 or SEQ. ID NO: 7.
6. A heparanase purified to apparent homogeneity, as in claim 3, prepared in the presence of reducing conditions, having an amino acid sequence of SEQ. ID NO: 1.
7. A heparanase purified to apparent homogeneity, as in claim 3, prepared in the presence of reducing conditions and activated with transglutaminase, having an amino acid sequence of SEQ. ID NO: 3.
8. A heparanase according to Claim 4, purified to apparent homogeneity, prepared in the presence of reducing conditions and activated with transglutaminase.
9. A heparanase, as in claim 3, prepared by recombinant means, activated with transglutaminase and having an amino acid sequence selected from the group consisting of SEQ.

ID NO: 1, SEQ. ID NO: 3, SEQ. ID NO: 5 or SEQ. ID NO: 7.

10. A heparanase, as in claim 3, prepared by recombinant means, activated with transglutaminase and having an amino acid sequence of SEQ. ID NO: 1.

5

11. A heparanase, as in claim 3, prepared by recombinant means, activated with transglutaminase and having an amino acid sequence of SEQ. ID NO: 3.

12. A heparanase purified to apparent homogeneity, as in claim 3, prepared in the presence
10 of reducing conditions and activated with transglutaminase, and having an amino acid sequence selected from the group consisting of SEQ. ID. NO: 12, SEQ. ID. NO: 14; SEQ. ID. NO: 16, SEQ. ID. NO: 18, SEQ. ID. NO: 20, SEQ. ID. NO: 22 and SEQ. ID. NO: 24.

13. A heparanase purified to apparent homogeneity, as in claim 3, prepared in the presence
15 of reducing conditions and activated with transglutaminase, and having an amino acid sequence selected from the group consisting of SEQ. ID. NO: 26, SEQ. ID. NO: 28, SEQ. ID. NO: 30, SEQ. ID. NO: 32; SEQ. ID. NO: 34, SEQ. ID. NO: 36, SEQ. ID. NO: 38 and SEQ. ID. NO: 40.

14. A method according to claim 1 wherein the heparanase is purified to apparent
20 homogeneity, prepared in the presence of reducing conditions and activated with transglutaminase, and having an amino acid sequence selected from the group consisting of SEQ. ID. NO: 12, SEQ. ID. NO: 14; SEQ. ID. NO: 16, SEQ. ID. NO: 18, SEQ. ID. NO: 20, SEQ. ID. NO: 22 and SEQ. ID. NO: 24.

25 15. A method according to claim 1 wherein the heparanase is purified to apparent homogeneity, prepared in the presence of reducing conditions and activated with transglutaminase, and having an amino acid sequence selected from the group consisting of SEQ. ID. NO: 26, SEQ. ID. NO: 28, SEQ. ID. NO: 30, SEQ. ID. NO: 32; SEQ. ID. NO: 34, SEQ. ID. NO: 36, SEQ. ID. NO: 38 and SEQ. ID. NO: 40.

30

16. A method according to claim 1 wherein the heparanase is purified to apparent homogeneity, prepared in the presence of reducing conditions and activated with transglutaminase, and having an amino acid sequence selected from the group consisting of
35 SEQ. ID NO: 1, SEQ. ID NO: 3, SEQ. ID NO: 5 or SEQ. ID NO: 7.

17. A peptide having an amino acid sequence of SEQ. ID. NO: 42.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 94/08207

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/34 C12N9/24 C12N9/96 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ANALYTICAL BIOCHEMISTRY, vol.157, no.1, 15 August 1986, NEW YORK US pages 162 - 171 MOTOYO NAKAJIMA ET AL. 'A Solid-Phase Substrate of Heparanase: Its Application to Assay of Human Melanoma for Heparan Sulfate Degradative Activity' see the whole document ----	1,2
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol.259, no.4, 25 February 1984, BALTIMORE, MD US pages 2283 - 2290 MOTOYO NAKAJIMA ET AL. 'Metastatic Melanoma Cell Heparanase' see the whole document	1,2
A	see page 2289, left column, line 63 - right column, line 31 ----- -/-	3,4

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

9 December 1994

Date of mailing of the international search report

21.12.94

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INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/US 94/08207

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,91 02977 (HADASSAH MEDICAL ORGANIZATION) 7 March 1991 cited in the application see page 23, paragraph 6.1.5 - page 26, paragraph 6.1.7; claims 1-3 ---	3-16
A	FASEB JOURNAL, vol.5, no.15, December 1991, BETHESDA, MD US pages 3071 - 3076 CHARLES S. GREENBERG ET AL. 'Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues' cited in the application see the whole document ---	3-16
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol.265, no.28, 5 October 1990, BALTIMORE, MD US pages 17180 - 17188 ELEONORA CORDELLA-MIELE ET AL. 'A Novel Transglutaminase-mediated Post-translational Modification of Phospholipase A2 Dramatically Increases Its Catalytic Activity' cited in the application see the whole document ---	3-16
A	WO,A,93 09794 (UNIVERSITY OF PENNSYLVANIA) 27 May 1993 see especially Sequence Description: SEQ ID NO: 1 see page 17, line 1 - line 16 -----	17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/08207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9102977	07-03-91	AU-B- 654804	24-11-94
		AU-A- 6336490	03-04-91
		CA-A- 2065744	24-02-91
		EP-A- 0487627	03-06-92
		JP-T- 5504047	01-07-93
		US-A- 5362641	08-11-94
WO-A-9309794	27-05-93	AU-A- 3071492	15-06-93

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